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I.O.L.R. REPORTS

A

FINAL REPORT

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B. Rinkevich

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FINAL REPORT

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Costa Rican coral reefs under siltation stress

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Grantee Institution: IOLR

Collaborator: Jorge Cortes

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Table of Contents

Executive Summary

The major aim of this collaborative work (Project C14-015) is to evaluate the effects of siltation stress on Costa Rican coral reefs, to determine stress phenomena, to restore denuded reef areas and to train young Costa Rican scientists in reef management and restoration. In addition, one of the major goals of the project is to transfer modern techniques and knowledge in coral reef biology and in the biology of reef organisms to Costa Rican scientists.

During the course of the project, several lines of research have been conducted in the field and in the laboratory. In the field, both groups, (the Costa Rican group is headed by Dr. J. Cortez, and the Israeli group is headed by Dr. B. Rinkevich) continued the collaborative monitoring studies in the reef on the Pacific Coast of Costa Rica (once a month) and on the Caribbean Coast (every 3-4 months). Long-term monitoring observations of specific selected stations were followed at the same pace along the research duration, in addition to field studies on variety of stress phenomena, such as the appearance of tumors in coral tissues and skeletons, bleaching events in Costa Rican reefs, interspecific and intraspecific competitions, and more. We also analysed fragments (ramets) and nubbins as a source materials for restoring denuded coral reefs. Fragmentation in scleractinian corals has been recognized as an important facet in the life history portrait of many species. Ramets isolated from existing genets may establish new colonies; a phenomenon that is widely used in a variety of management measures. An analysis of regenerating branches of a branching coral reveals that the architectural complexity of isolated branches may have a significant impact on the initiation of the regeneration process towards the typical structure and complexity of an intact colony. It was revealed that the 3-D structure complexity of isolated ramets should be taken into consideration when applying reef restoration practices. We also tested during this period several strategies for gardening denuded reefs. The gardening concept consists of *in situ* or *ex situ* mariculture of coral recruits, followed by their transplantation into degraded reef sites. *In*

situ nurseries were established in shallow waters, sheltering three types of coral materials taken from branching coral species (small colonies, branch fragments, and spat) that were monitored for up to two years. Pruning more than 10% of donor colonies' branches increased mortality and surviving colonies displayed reduced reproductive activity. We found that maricultured isolated branches, however, exceeded donor colony life span and reproductive activity. Nursery periods of 2 years, 4-5 years and more than >5 years have been estimated for small colonies, spat, and isolated branches, respectively.

Both groups mutually carried out the study. To further facilitate the work, two Israeli scientists (Dr. D. Gateño and Dr. Y. Barki) have resided in Costa Rica, participating in all field trips and other scientific activities involved in the project. The Israeli PI visited Costa Rica one to two times/year for tight collaboration and discussions with the Costa Rican group. Funds were also used for participation of the Costa Rican partner, Jorge Cortés, in the IX Congreso Latinoamreicano sobre Ciencias del Mar (COLACMAR), San Andrés, 16-20 September, 2001 (Latin American Congress on Marine Sciences). The project results were presented in this symposium by two oral presentations. In addition, scientists from Costa Rica visited the laboratory at Haifa and participated in field studies at Eilat. Eleven publications have already been published from this study (three others are in press, an additional two publications are in preparation).

RESEARCH ACTIVITIES

The proposed study was planned to be conducted in Costa Rica and Israel through the following four main routes:

1. Long-term determination of suspended and re-suspended sediment in the studied areas
2. Evaluation of siltation stress on the population and colony levels

3. Restoration of sediment-stressed coral reef and denuded areas by:
    a. Coral fragments
    b. The use of sexual recruits both by transplantation of gravid colonies to denuded areas and by newly metamorphosed larvae collected and settled in the laboratory
4. Training of Costa Rican scientists in reef management, histology and histopathology of corals.

Field stations were established on both sides of Costa Rica: in Parque Nacional Cahuita (the Caribbean Sea) and in the Pacific Ocean. Both areas are heavily stressed by siltation. Since both areas contained live, flourishing reefs, partially dead and completely dead reefs, all comparisons can be made within the same selected area without being involved in comparisons of areas with significantly different environmental conditions. For that purpose, we established long-term monitoring photo station surveys, evaluated several stress phenomena (in addition, characterized by molecular study the sequence of the first stress protein, HSP-70, from a coral species) and followed, in a detailed study the appearance of tumors in coral colonies. Field stations also revealed quantitavely the load of sedimentation in Costa Rican reefs.

A major part of the research activities (also revealed in the list of publications published under this AID-CDR project) was involved with the rationale of active rehabilitation of reefs under stress and the use of coral mariculture. We consider the coral mariculture concept as a major component in reef restoration that has been so far neglected. This strategy may form the conceptual and practical platform for reef restoration activities. In comparison to the harmful practice of harvesting corals for transplantation from donor reef areas, the establishment of coral nurseries, containing local species and genets that are managed in a sustainable manner, eliminates the need for the extraction of valuable coral material for transplantation. A protected nursery phase provides the transplanted material with an acclimation period for

better survivorship and growth to a size suitable for transplantation. The transplantation of nursery-grown 'propagules' back into their natal reef helps in preventing genet and species extinctions in degrading sites, exercising the 'rescue effect' on a local scale. It also preserves the genetic heterogeneity. A coral nursery may also be considered as a local species pool that supplies reef-managers with coral colonies for sustainable management.

The coral mariculture concept incorporates the implementation of *ex-situ* as well as *in-situ* nursery techniques. Whole small colonies and ramets may be directly placed in low profile structures attached to the reef bottom, designed to provide a firm but temporary structure. Upon reaching a suitable size and shape (depending on the species' morphometric codes and its capacity to regenerate and reconstruct the typical colonial architecture), cultivated colonies are transplanted back into their natal reef in carefully planned transplantation programmes. Where applicable, nubbins and planulae may be kept first for initial growth period under *ex-situ* controlled conditions prior to their transfer to *in-situ* nurseries. During the course of this project, we have revealed that coral mariculture is an essential, complementary component for reef restoration, in conjunction with transplantation techniques.

During the course of the proposed study, we were also engaged with extensive training of Costa Rican scientists by both, visits to the Israeli laboratory and mainly, by having two Israeli scientists residing in Costa Rica. The two scientists (Dr. D. Gateño and Dr. Y. Barki) were actively engaged in the research in Costa Rica, trained local M.Sc. students and technicians and were involved in decisions for future activities in coral reef restoration in this region.

The scientific results of this project have been published and were accepted for publication in 14 peer-reviewed manuscripts (detailed in "Project Activities/Outputs, p. 100). Results of ten of these publications were outlined in details in former annual reports and will

not be repeated here. The other four types of activities (including additional outcomes [in preparation]) will be outlined in the next chapter, presented as if for publication in a scientific journal.

RESULTS

The six activities detailed below are associated with two of the major aims of the research, the questions involved with restoring denuded coral reefs and the evaluation of stresses/diseases found in the areas that suffered from intensive human activities.

1. Forest restoration principles and reef restoration practices.

Two Ecosystems With A Similar Fate

Trees and stony corals, the basic framework building blocks of rainforests and coral reefs, share a common ecological role by virtue of their biological properties (Yonge, 1973; Connell, 1978; Goreau *et al.*, 1979; Christensen *et al.*, 1996). By being the primary habitat constructors, these sessile organisms not only bear similarities in their contribution to the ecosystems' structural arrangement, but also follow similar basic architectural rules in their growth and pattern formation characteristics (Dauget, 1991). Reef corals and rainforest trees also harbour numerous inhabitants and provide the ecosystem with the structural design and strength to resist physical disturbances such as storms.

In spite of continuous exposure to destructive environmental agents, coral reefs and rainforests have successfully persisted over evolutionary time scales (Lugo *et al.*, 2000). This has been achieved through mechanisms of resistance and fast structural repair of their prime components. Natural disturbances that clear up reef and rainforest areas promote seed bank germination or coral larvae settlement (Lorimer and Frelich, 1994; Lugo *et al.*, 2000). Re-colonization marks the onset of succession within the impacted area that will eventually restore species composition and physical structures unless further disturbed. In chronically

stressed situations, however, as is with most tropical forests and coral reefs worldwide, disturbances may alter the ecosystems to a point that natural resilience is hindered.

Coral reefs and rainforests have been facing dramatic threats from new types of perturbations during the last five decades, as damaging human activities rapidly increased in scale and intensity. Forest clearing and land conversion to agriculture leads to soil erosion, and desertification. Similarly, major declines in coral coverage due to man-made physical disturbances lead to the collapse of reef communities and the development of 'weedy' algal mats (Kinsey, 1988; Lugo *et al.*, 2000). As tree logging is recognized as the major destructive agent of forest areas (Dobson *et al.* 1997; Noble and Dirzo, 1997), marine-based recreational activities fulfill this role in the coral reefs (Chadwick-Furman, 1997; Muthiga and McClanahan, 1997). As a result, both ecosystems undergo dramatic changes in structure and species composition under regimes of chronic human impacts (Loya, 1986; Rinkevich, 1995; Noble and Dirzo, 1997), displaying diminishing capacities to ecological resilience, to absorb disturbances, to reorganize and to adapt to changes.

On land, the failure of forest systems to self-regeneration has stimulated the development of various restoration measures that have been proven to be effective (literature cited in Putz *et al.*, 2001). These restoration acts have succeeded in reversing the trend of deterioration and creating new habitats for biodiversity (Dobson *et al.*, 1997). This is not the situation with coral reefs (Allison *et al.*, 1998) where the effectiveness of rehabilitation measures that have been employed to date, such as artificial reefs and coral transplantation, is debatable (Edwards and Clark, 1998). As a matter of fact, in the coral reef arena, no single restoration concept is commonly accepted. We deal here with the question of whether approved forest restoration concepts can be adapted and utilized for reef restoration as well.

RESTORATION ECOLOGY VS. CONSERVATION BIOLOGY

Both terms "restoration" and "conservation" are often used in the context of efforts aiming to "preserve" the original habitats or to "replace equivalent" lost habitats and destroyed populations. However, restoration ecology bears the notion of active measures while conservation biology focuses on "passive" measures, allowing natural processes to mitigate impacts without or with only minimal human interference. Restoration ecology has therefore at its core the assumption that at least some proportion of habitat loss is recoverable through positive action. A recent review (Young, 2000) revealed that while conservation biology has been far more biased with respect to the zoological aspect (by nearly 3 to 1 in the number of manuscripts published), restoration ecology has been far more biased with respect to the botanical aspect (by more than 4 to 1). A similar botanical bias has also been noted for other applied ecological concepts such as the use of the "wilderness" concept in marine conservation (Sloan, 2002). The development of a restoration agenda to coral reefs, therefore, could benefit from the more advanced and organized concepts existing in the botanical arena. A robust restoration framework for the coral reef environment should equally set its priorities as the enhancement of reef biodiversity and the maintenance of ecosystems' critical functions thus providing the platform on which sustainable reef restoration should progress.

TERRESTRIAL RESTORATION CONCEPTS

Habitat restoration is a scientific discipline developed originally for terrestrial ecosystems (Hobbs and Norton, 1996). Its rationale operates at two distinct hierarchical levels, the first of which is to restore the physical environment, followed by the re-introduction of vegetation. Several working concepts guide these restoration protocols. They include the need for preservation of critical ecosystem functions (such as nutrient and mineral cycling), the priority handling with key species, the need for preserving biological diversity, the importance of rehabilitation of biological framework structures and the impacts on human welfare

(Christensen *et al.*, 1996; Dobson *et al.*, 1997; Lesica and Allendorf, 1999; Köhlin and Ostwald, 2001; Putz *et al.*, 2001). In practical terms this translates to a restoration strategy aiming to ameliorate the situation of trees, the key species populations. This, in turn, may result in the enhancement of biodiversity and the maintenance of ecosystems' critical functions. These concepts underpin the practice of forest restoration.

In many areas of the world, the dependency on forest products is significant. Increased pressure for timber, fuel wood, cropland and commercial industry has had a direct impact on forests worldwide. Degraded forestlands are further prone to land erosion, decreased hydrological stability, lower primary productivity, and diminished species diversity. Re-forestation of partially degraded areas has been suggested to be the most practical measure (Köhlin and Ostwald, 2001) and is achieved through several routes, with the prime aim to maintain species composition and diversity (Dobson *et al.*, 1997). Areas of advanced deprivation, or extinct forest communities are reconstructed through silviculture programs by cultivating selected tree species in plantations. Several tactics to achieve successful silviculture include the choice of species that deliver the highest performance in restoration situations, the use of local genotypes (when available), the use of phenotypically plastic species with wide ecological amplitudes, the sequential introduction of species, the restoration of substrate/landscape components of the ecosystem, and more (Dobson *et al.*, 1997; Lesica and Allendorf, 1999). Since the above activities have effects on ecological as well as on plant population parameters, all sivicultural decisions necessarily represent compromises between science and pragmatism (Table 1; Dobson *et al.*, 1997; Putz *et al.*, 2001). Nevertheless, silviculture is considered a key strategy to achieve objectives at the community level, such as enhancing productivity, stability and biodiversity of denuded areas (Parrotta, 1992), and the concept has proven to be one of the most promising restoration tools for tropical forest systems (Christensen *et al.*, 1996).

Due to the biological structural and functional analogies between trees and corals, it is natural to propose that silviculture concepts can assist in reorganizing the theoretical and practical priorities for coral reef restoration so that a solid restoration framework can be developed for the marine realm. The strategy of coral mariculture and gardening may potentially prove to be a sustainable practice for reef restoration, comparable to silviculture (major points for comparison are outlined in Table 1). The measure for "gardening the coral reef" is an improved strategy to the more traditional and widely used measures of "coral transplantation", where the need to harvest coral colonies from existing populations represents a major imperfection.

CORAL TRANSPLANTATION MEASURES

During the last decade, coral transplantation operations have been frequently employed following acute mechanical disturbances that denude reef areas and have gained recognition as the prime tool for reef restoration. Various coral transplantation techniques are widely used in attempts to restore degraded reef areas, such as the use of coral nubbins, fragments and whole colonies (literature cited in Harriott and Fisk, 1988; Edwards and Clark, 1998; Lindahl, 1998; Franklin *et al.*, 1998; Bruckner and Bruckner, 2001; Bowden-Kerby, 2001; Table 1). The rationale behind the practice of coral transplantation is to replace dead coral colonies in a devastated patch with new ones in order to speed up natural recovery. Early stages of natural succession may therefore be accelerated by transplantation of gravid coral units, which facilitates re-colonization (van Treeck and Schuhmacher, 1997). This type of artificial intervention improves the state of diminished resilience of the ecosystem by reducing the course of biodiversity decline (Dobson *et al.*, 1997). However, several drawbacks are associated with the protocols of relocating healthy corals into denuded reef sites (Edwards and Clark, 1998). These include the high labour and financial cost of coral transplantation when large quantities are relocated from a donor area directly into the damaged site (Harriott and

Fisk, 1988), and the introduction of large amounts of alien material (Clark and Edwards, 1995). The harvesting of corals for transplantation disturbs undamaged reefs and inflicts stress on donor colonies. The damage to the source colonies is always weighed against the need of harvesting as many fragments as possible (Franklin *et al.,* 1998). Furthermore, transplantation leaves aspects of transplant survivorship and growth dependent on reef conditions at the damaged site and can thus be wasteful, since survivorship of corals that are transplanted directly upon harvest into a damaged site is often low.

The shift in frequency and magnitude of physical damage inflicted to reefs worldwide in the last decade from incidental to chronic and to intense, necessitates the development of a restoration strategy designed to cope with such a stressful state. This need is far more urgent in reef areas that are frequently visited by numerous water-sport devotees but are too small in area to implement efficient user-interface measures. In such cases (as at the coral reef reserve of Eilat, Red Sea; Rinkevich, 2000; Epstein *et al.*, 2001), or in other acute situations, reef managers should be able to provide transplantation operations with local coral genotypes harvested from a managed coral pool. In none of the currently prevailing coral transplantation methods was this concept considered. Transplantation operations are normally performed on a demand basis following wide-scale disturbances, and as such are the cause of additional damage to donor areas.

Whilst physiological and morphological considerations such as variables in growth rate and survivorship are taken into account in transplantation exercises, aspects of pattern formation, as dictated from initial fragment size and its structural complexity have been neglected. Similarly, the ability of some coral species to express a variety of ecomorphs (phenotypic plasticity, the morphological response to environmental conditions) has not been considered thoroughly in reef restoration (Epstein and Rinkevich, 2001; Rinkevich, 2002). The morphometric codes of initial fragment architectural complexity and phenotypic

plasticity are important factors in the practice of coral mariculture (Epstein and Rinkevich, 2001).

An improved strategy for reef restoration is the "coral gardening approach" (Rinkevich 1995, 2000), where isolated ramets, nubbins and spats are continuously cultured *in-situ* in protected underwater nurseries or are reared to begin with *ex situ* in inland nurseries before transferring them to *in situ* conditions. Such an approach is based on the concepts developed for forest restoration and on the already existing outcomes for coral mariculture.

CORAL MARICULTURE

We consider the coral mariculture concept as a major component in reef restoration that has been so far neglected. This strategy may form the conceptual and practical platform for reef restoration activities. In comparison to the harmful practice of harvesting corals for transplantation from donor reef areas, the establishment of coral nurseries, containing local species and genets that are managed in a sustainable manner, eliminates the need for the extraction of valuable coral material for transplantation (Rinkevich, 1995, 2000; Epstein and Rinkevich, 2001; Epstein *et al.*, 2001). A protected nursery phase provides the transplanted material with an acclimation period for better survivorship and growth to a size suitable for transplantation. The transplantation of nursery-grown 'propagules' back into their natal reef helps in preventing genet and species extinctions in degrading sites, exercising the 'rescue effect' on a local scale (Sinsch, 1997). It also preserves the genetic heterogeneity. A coral nursery may also be considered as a local species pool that supplies reef-managers with coral colonies for sustainable management (Rinkevich, 1995, 2000; Epstein *et al.*, 2001).

The coral mariculture concept incorporates the implementation of *ex-situ* as well as *in-situ* nursery techniques. Whole small colonies and ramets may be directly placed in low profile structures attached to the reef bottom, designed to provide a firm but temporary structure. Upon reaching a suitable size and shape (depending on the species' morphometric

codes and its capacity to regenerate and reconstruct the typical colonial architecture), cultivated colonies are transplanted back into their natal reef in carefully planned transplantation programmes. Where applicable, nubbins and planulae may be kept first for initial growth period under *ex-situ* controlled conditions prior to their transfer to *in-situ* nurseries. Coral mariculture is an essential, complementary component for reef restoration, in conjunction with transplantation techniques.

Much of the literature on ecological restoration pertains to the choice of species to be used (Lesica and Allendorf, 1999). Taxa used for restoration are not selected at random but often conform to the definition of 'key species; i.e., they perform a function that is vital for the ecosystem. Selected coral species for mariculture are often good representatives of local common coral species and in many cases, are of the branching coral forms (Bowden-Kerby, 1997, 2001; Franklin *et al.,* 1998; Epstein *et al.,* 2001). Branching forms are also selected for their high performance in restoration situations (i.e. survivorship and growth rates). Branching coral colonies potentially provide several types of coral material for mariculture, including ramets (incorporating a single or several branches), nubbins (fragments the size of a single or several polyps), small whole colonies removed from shallow, frequently disturbed areas, where long-term survival is unlikely, and planula larvae (Rinkevich, 1995, 2000; Bowden-Kerby, 2001; Epstein *et al.,* 2001). As mentioned, *ex-situ* nurseries (accommodating minute nubbins and settled coral planulae) are used only for a preliminary foster period, before transferring the cultured material to the *in-situ* nurseries. Viable ramets and spat are known to be able to survive in protected *in-situ* and *ex-situ* nurseries long after their parental colonies have succumbed as a result of stress from human impacts on their original habitats (Epstein *et al.,* 2001).

Several studies have already successfully tested aspects of coral gardening techniques (Clark and Edwards, 1995: Bowden-Kerby, 1997, 2001; van Treeck and Schuhmacher, 1997;

Franklin *et al.,* 1998; Rinkevich, 2000; Bruckner and Bruckner, 2001). For example, Bowden-Kerby (1997) demonstrated the potential of a sheltered, lagoon-like reef area to be used as a natural nursery for loosely scattered corals. Franklin *et al.* (1998) successfully cultured coral fragments *in-situ,* cemented into small plastic cups, and Rinkevich (2000) demonstrated the potential of low profile substrates as nurseries. Epstein *et al* .(2001) have estimated a nursery phase as short as 2 years for ramets of the Indo Pacific branching coral species *Stylophora pistillata.* This minimal mariculture phase was achieved through combining *ex-situ* and *in-situ* farming of metamorphosed, fragments and small colonies, that took into consideration pattern formation processes (Epstein and Rinkevich, 2001; Rinkevich, 2002).

SUMMARY

Direct human intervention is necessary to restore degraded reef habitats and active coral reef restoration is beginning to be viewed as a necessary measure (Bowden-Kerby, 2001). Any successful reef restoration methodology should be appropriately adapted to local socio-economic limitations and be subjected to insular landscape conditions. The gardening concept, however, may be applied to different reef locations worldwide subject to site-specific considerations and may serve as a ubiquitous rationale to reef restoration (Rinkevich, 1995, 2000). Management decisions should take into consideration the appropriate target coral species, and the type of source material most suitable for local cultivation. Sustainable coral gardening and transplantation programs may support and enhance the existence of resident coral taxa and prevent further deterioration of the entire reef community. The gardening concept may contribute to the formation of a meaningful reef mitigation framework: conservation of threatened coral species through mariculture, and rehabilitation through their transplantation.

Ecosystem management, in terrestrial and marine habitats alike, is associated with social, political, economic, cultural, and ecological themes (Christensen *et al.,* 1996; Thomas, 1996).

For example, the decision as to which habitat should be restored may be as important as to how much is to be restored. Non-random restoration practices such as restoring only a habitat that is adjacent to those occupied by the target species can dramatically reduce or negate any restoration lag (Huxel and Hastings, 1999). Moreover, in many cases of forest management, silvicultural practices are designed to promote the regeneration and the stocking of commercial species for timber forest products (Putz *et al.*, 2001; Table 1). This is not the case with coral reefs. In economically important reef sites (such as for the tourist industry), the coral mariculture strategy is used to develop sustainable material for human and non-human stakeholders, in a way that all components of reef habitats benefit. The restoration of coral reefs, therefore, should become a standard part of conservational practices, and when applied, already tested and approved forest restoration principles may provide important insight into the understanding of the reef ecosystem recovery.

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Table 1. Comparing key criteria for forest and coral reef restoration measures (common elements for both ecosystems are marked with black dots)
Sources: Chadwick and Stephens, 1977; Connell, 1978; Goreau *et al.*, 1979; Loya, 1986; Kinsey, 1988; Johnson and Preece, 1992; Parrotta, 1992; Rinkevich, 1995, 2000; Christensen *et al.*, 1996; Lesica and Allendorf, 1999; Lugo *et al.*, 2000; Epstein and Rinkevich, 2001; Epstein *et al.*, 2001; Putz *et al.*, 2001.

Points for consideration	Criterion	Building Stones	
		Trees	Corals
Plantations and nurseries	Source material for farming	Asexual: ramets Sexual: seeds, seedlings	Asexual: ramets, nubbins Sexual: Planula larvae, spat
	Location	Selected sites for plantations or directly on degraded forest land	In-situ nurseries combined with ex-situ aquaculture
	Type	Monoculture or mixed species plantations	Mariculture of local key coral species
	Rationale	• No detrimental impact on environment • Greenhouse needed for sustainable number of recruits • Nursery survivorship is higher than in under natural conditions • Stock material is available upon immediate request	
	Species of choice Decisions	• Direct controlling of species abundance • High performance in restoration situations • Necessarily represent compromises for species used, locations, measures, etc.	
	Time for transplantation	Several years	As soon as two years from initiation
	Purposes	In many cases, stocking of commercial species	To revive denuded areas
Outcomes	Immediate target	Young trees to cover land in denuded sites	New coral colonies to replace dead corals or create new nuclei in denuded zones
	Ecological properties	• Facilitating subsequent recruitments, preventing takeover by weedy species	
	Impact on substrate	Root system: stabilizing soil Enhanced litter production; increased soil organic matter, moderating soil pH, and improving nutrient status	Enhanced lithification processes Enhanced reef rock formation
	Overall ecological impact	• Restoring productivity, stability and biodiversity • Restoring associated fauna and flora • Increasing spatial resilience of ecosystems • Speeding the recovery of degraded habitats	

2. Colony pattern formation: impacts on restoring coral colonies.

Modules and Plasticity

One of the central questions in developmental biology is the issue of how different species evolve different morphologies (Purugganan, 1998) and within a given species, how conspecifics exhibit distinctive and diverse morphological features. The ability of organisms to produce different phenotypes under different environmental and biological challenges and conditions (a phenomenon termed as "phenotypic plasticity") is one of the fundamental aspects of developmental biology. A number of studies in the past few years have demonstrated the existence of "plasticity genes" that specifically respond to a particular type of environmental alteration by triggering a of morphogenic change (Pigliucci, 1996).

Some species represent high phenotypic plasticity. However, even in such cases, there is also the possibility that a stringent species-specific "morphometric code" applies. Such a code is composed of a set of rules common to processes that are used repeatedly and in different combinations, to make living organisms functional under variable environmental conditions. In other words, the elements that construct an organism (animal, plant, etc.), are heritable characteristics of each species. One such example is the geometrical body invariance known as symmetry (Goldstein and Freeman, 1997; Levin, 1997; Martindale and Henry, 1998).

A variety of animals, mainly aquatic sedentary sessile organisms (sponges, corals, hydrozoas, bryozoans, tunicates) which are made up of modular units (usually called "polyps" or "zooids"), cumulatively form structures termed as "colonies". Colonies are therefore a tessellation of their structural modules. Colonial animal modules may vary significantly in size, architecture, arrangement in space, pattern, integration, and mode of development (Jackson and Coates, 1986; Tuomi and Vuorisalo, 1989; Pedersen and Tuomi, 1995). I accept the idea that a compound organism with colonial architecture does not represent an aggregate of individuals, but instead may be regarded as a single organism possessing characters of

morphological and physiological integrity. This is based on Beklemishev (1970) suggestion of the "balance" between a gradual increase in colonial individuality versus a simultaneous decrease in individuality of the zooids in some colonial organisms and the notion of the unit-of-selection status (Rinkevich, 2000a). The modular idea is essentially a visual (morphological) and topological one (Rosen, 1986), and is hierarchically constructed on up to three levels of organization: primary modules or zooids; modules of the second order, grouped zooids arranged in replicated patterns (may be called branches, such as branching corals or "systems", as in botryllid ascidians); and third order modules, the daughter colonies or ramets (Ryland and Warner, 1986). With regard to body construction, the first and the third order modules (the zooid and the ramet) and the uppermost organizational level, the genet, have captured most scientific interest.

Colonial structures emerge as iterative processes of successive layers of material (Kaandrop and de Kluijer, 1992). In branching colonial organisms, morphology is established through iteration of two structural units: modules of the first order (the zooids) and modules of a second order (the branches; in many cases representing ramet level). Variations in the branching morphologies of colonial organisms are frequently correlated with a suite of life-history traits (Buss and Blackstone, 1991). A significant portion of relevant literature examines correlations between environmental qualities and morphometric analyses (either on the zooid, ramet or genet level; Chamberlain and Graus, 1975; Bottjer, 1980; Sebens, 1984; Abelson et al., 1991; West, et al., 1993; Helmuth et al., 1997; Johnson, 1997; Vago et al., 1998 and literature therein). While there is no doubt that environmental factors may tune phenotypic architecture, the common high fidelity of morphological structures reveals development homeostasis, probably controlled by the genetic background of the species of interest.

THE RATIONALE FOR COLONY ARCHITECTURE

Descriptions of colony growth can be supported by simple characterization of the rate at which new modules are generated, or can be based on the architecture and the 3-D structures that emerge over time (Prusinkiewicz, 1998). Inherited traits in colony forms and the iterative processes which shape colonial structures, may suggest that colonial architecture is the measurable outcome of developmental homeostasis pathways. Even the expression of high phenotypic plasticity, the outcome of the interactions between genotypes and heterogeneous environments (Callahan et al., 1997), may be rooted in the genetic background of the organisms and, as a result, can be evaluated by measurable codes and patterns. The study of colonial architecture, therefore, relies on the perception that colonial organisms are integrated wholes, rather than structural collections of modules (Rinkevich, 2000a), and compares changes in structures with an eye to a better understanding of heritable traits (Nijhout et al., 1986).

Different approaches to modeling colonial organization can be arranged within a polarized continuum. At one end is the idea that pattern formation of a colony is a morphologically rigid intrinsic process and genetically controlled. It is a centralized phenomenon working on the colony (genet) level. As a result, the responses to environmental factors are achieved through the establishment of ecological races, genetically different ecotypes. At the other end is the idea that colonial form is strictly flexible, shaped by trade-offs between different traits. Therefore, defining architecture is the science of defining a suite of characters that respond discretely to the environment. According to this notion, "variations" is the conserved trait. Surprisingly enough, there are documented observations that even such phenotypic plasticity phenomena are probably also controlled by genetics, specifically by plasticity genes (Pigliucci, 1996; Callahan et al., 1997). One major pitfall of "trade-off" approaches is that

many of them share the trait of not describing colony growth with explicit explanations for the processes that control colony allometry.

With regard to a branching system, the description of the organism in modular terms, the most commonly used illustration, is not too difficult. However, not recognizing interactions between colonial components may present some theoretical and practical difficulties (Bell, 1986). Since the implication of geometrical forms implies the use of mathematical tools, the rationale and assumptions based on the mathematical models employed are key issues in understanding the outcomes. For example, a variety of models used for development, including the formal one-dimensional algorithms (reviewed in Nijhout et al., 1986), did not address, and therefore missed, the possible relationship between gene expression and morphological features. The same problem applies to models developed by biologists which assume that morphologies are strictly determined by environmental features such as the availability of food particles (Abelson et al., 1993; West et al., 1993; Vago et al., 1998; and literature therein), although other studies (e.g., Amaral, 1994) clearly point out that changes in morphology can not be explained solely by environmental variables.

More and more studies in the last two decades suggest an intrinsic order to branched growth, even in the most elaborate systems (Bell, 1986), although few have explicitly considered the problems and constraints posed by ontogeny on forms (Waller and Steingraeber, 1985). One such approach was to apply the "tree architectural models" to coral growth forms (Dauget, 1991a,b, 1994, and literature therein). These comparisons revealed common rules for branching and ramifying axes systems, for the organization of these axes in identical architectural models, for the reiteration pattern in the course of growth and also for the physiognomic variations correlated with environmental parameters. Other approaches have been to adapt mathematical models such as fractals and simulations of diffusion and flow (Waller and Steingraeber, 1985; Kaandorp and de Kluijver, 1992; Kaandorp and Sloot,

1997; Kaandorp, 1999; and literature therein) to branching patterns of colonial marine invertebrates. The above approaches were mainly employed by mathematicians.

Other approaches have been employed mainly by evolutionary biologists. One of these is the adaptation of Waddington's (1942) theory regarding morphogenesis of colonial organisms (Buss and Blackstone, 1991). According to this approach, the development of a colony is represented as a problem in a dynamic system theory where colony reactions to environmental challenges are canalized; such adapted reactions result in one definite end point, regardless of minor variations in conditions during the course of development (Waddington, 1942; Buss and Blackstone, 1991). A second approach is to adapt the concept of heterochrony (the change in timing of development) to characterize colonial organisms as integrated wholes rather than as atomistic traits (Blackstone and Yund, 1989; Blackstone and Buss, 1993). From this perspective, alterations of development during time-windows are analyzed as heterochronous changes intrinsic to the organism. However, since the study of heterochrony is based on comparable values (Blackstone and Yund, 1989), it is not equipped to reveal the biological and genetic bases for pattern formation. In addition, not all pattern-formations are age-based measurements. None of the above approaches, however, address the relationships between gene activities and morphological features (Nijhout et al., 1986).

BRANCHING - THE NATURE OF COMPLEXITY

Branching forms of sessile marine organisms (well represented by a variety numbers of algae, sponges, cnidarians and bryozoans) are examples of morphological systems constructed from many simple identical components, which together are capable of developing complex structures. Such modular forms may be constructed in the regular manner of a high degree of organization, or maybe "bewilderingly complex and yet not haphazard" (Bell, 1986). Moreover, the existence of tissue connections between all modules in a branching colonial form, most probably allows the transmission of cues, molecules, energy resources and alarms

between the zooids (Mackie, 1986). Characteristics such as the sharing of resources, communication and coordination between remote parts of a branching structure (such as in the colonial hermatypic coral *Stylophora pistillata*, see next section) are therefore pre-requisites for colonial holism and fundamental attributes to "real" colonies, setting them apart from mere aggregates (Rinkevich, 2000a). Topics like growth patterns, senescence, and sexual vs. asexual reproduction (Jackson and Coates, 1986; Rinkevich and Loya, 1986; Rinkevich et al., 1992), are associated predictions with regard to colonial modularity. What has not been addressed in the relevant literature is the predicted genetic machinery, where highly specific and coordinated responses are evoked by arrays of regulatory genes acting at different hierarchical levels. One such example is the expression of homeobox genes (Miles and Miller, 1992; Cartwright et al., 1999).

A general colony form is schematically based on the lower level units (the zooids) and reveals two aspects: 1. components of organization, and 2. shape for every modular level presented. Branching organizations and branching shapes can occur at any modular level (Rosen, 1986). The higher level unit, the whole branched entity, has generally been used as the primary unit for ecological interaction considerations. Since modules (the zooids, and in some cases, the branches or the ramets), are the building blocks for colony organization, these integrated, semi-autonomous subunits should also be considered. Analysis of regenerating isolated branches of the branching coral *Stylophora pistillata* (see below) has revealed that initial architectural complexities of the ramets may have a significant impact on the regeneration processes towards the typical structure and complexity of an intact colony as a whole (Epstein et al., 2001).

There is yet no model system for the study of branching modular organisms that permits analysis of phenotypic plasticity from both the architectural and genetic trait points of view. Most data in the literature is fragmented and based on a variety of branching forms that reveal

different aspects of trade-offs among multiple traits, across multiple developmental levels and variable responses to different environmental challenges. I present here the branching scleractinian coral *Stylophora pistillata* as a model case study, illustrating the architectural beauty of this system. Part of this approach has been recently published (Rinkevich, 2001) and will be discussed here in brief. There are two working hypotheses: 1. that a *S. pistillata* colony represents the holistic structure, the functional unit of selection (*sensu* Tuomi and Vuorisalo, 1989; Rinkevich, 2000a), and 2. that coordination patterns are due to intrinsic, central colony level control (*sensu* Harvell, 1991; Cartwright et al., 1999). In other words, genetics is a key factor in shaping the colony landscape.

STYLOPHORA PISTILLATA: TRANSITIONS IN TACTICS

Stylophora pistillata (Esper, 1797) is a widely distributed Indo-Pacific branching coral species characterized by rapid growth rate and a variety of color morphs, from dark brown, purple and yellow, to pale pink. In the Gulf of Eilat (Red Sea), it is abundant in the lagoon, rear-reef and reef flats, and common in the fore-reef (Loya, 1976), down to 60m depth. Colonies of this species exhibit an axially rod-like growth form and each branch consists of numerous minute polyps (approximately 1 mm in diameter). *S. pistillata* is also an ecologically important key species forming a substrate and habitat for many species of crabs (mainly Xanthidae and Alpheidae), fish (especially Pomacentridae and Gobiidae), as well as variety of cryptic organisms such as sponges, bivalves, polychaetes and others.

The astogeny of a typical *S. pistillata* colony is characterized by well-controlled developmental processes. Primary polyps start to deposit calcareous skeletons about 1d following metamorphosis. One week thereafter, several additional polyps (usually six, similar to *Pocillopora*; Stephenson, 1931) are added extra-tentacularly from the peripheral tissue, to form a circle around the primary polyp. Growth rates of new polyps over time are highly variable among young colonies (Frank et al., 1997). This kind of lateral expansion continues

until at some, as yet an unidentified stage, branches develop by apical growth. Only a single apical ramified structure is developed from each "lateral plate". New structures are then added, developing in conformity with the basic architectural rules of this species. These structures are reiterated complexes. An overview of a well-developed *S. pistillata* colony clearly reveals that the space between the up-growing and side-growing branches (see below) tends to be filled by the reiterated complexes (Dauget, 1991a,b) avoiding direct tissue-to-tissue contact of different branches.

The resulting symmetry of a typical *S. pistillata* colony approximates a sphere (Loya, 1976). Within the volume of the sphere, up-growing branches (UGBs) are primarily added by dichotomous fission at a branch-tip (Rinkevich and Loya, 1985a; Rinkevich 2000b, 2001). The apex of each axis (UGB) comprises several contiguous polyps. As a result of fast growth of one of the new formed branches (Rinkevich, 2000b), apical ramification usually forms unequally sized axes. This is also reflected by the measured high (70%) variation in mean growth rates between all UGB tips of individual *S. pistillata* colonies. Tip growth ratios within newly formed dichotomous UGBs differ significantly from older branches, further emphasizing the within-colony genetic background for spatial configuration (Rinkevich, 2000b). In addition to the UGBs, many lateral, inward and outward facing branches (LBs) are formed. Outward-facing LBs elongate similarly to UGBs, adding further lateral volume to the colony's spherical structure (Loya, 1976). With regard to inward LBs, it could be predicted that after prolonged elongation they would encounter and fuse with UGBs. However, such fusing was never observed in intact *S. pistillata* colonies since these branches cease to grow at a certain point (Rinkevich and Loya, 1985a). The decrease in growth rates of inward LBs, the change in growth directionality of isogenic branches that risk continguity (Rinkevich and Loya, 1985a), the lack of fusion between branches of a colony, and the retreat growth occasionally recorded between closely growing branches of allogeneic colonies (Rinkevich

and Loya, 1985b), reveal the existence of feedback mechanisms that "consider" current shapes and future growths within an architectural scheme of preplanned pattern formation. The possible appearance of chemical signals carrying biological activities that control these growth patterns has been suggested (Rinkevich and Loya, 1985a).

Following the expression of genetic components, internal transport of signals and nutrients may have strong impacts on pattern formation and allometry of many modular taxa, including *S. pistillata*. For example, translocation of photosynthates is recorded when *S. pistillata* colonies are allogeneically grafted with ^{14}C labeled branches. The host colonies translocate the labeled photosynthates towards the regenerating portions (Rinkevich and Weissman, 1987), probably through the gastrovascular canals that connect different polyps. Some of the daily fixed metabolites are stored within the colony for future use in other parts or biological compartments. Planula-larvae, collected 1-7 months after the coral tissue was labeled with ^{14}C, were found to contain significant amounts of labeled photosynthates (Rinkevich, 1989). Similarly, photosynthetically fixed products of a specific single day were still found to be contributing to newly formed tissues, months after labeling (Rinkevich, 1991). Reproductive activities are also developed and shaped at the colony level (Rinkevich and Loya, 1979), as are aging processes. In old, senescent colonies, reproductive activities and calcification rates decreased synchronically in all branches. New and old polyps exhibited senescence simultaneously, leading to complete tissue mortality (Rinkevich and Loya, 1986). Together, these findings reveal that *S. pistillata* (probably as in other branching colonial organisms) possess conserved "morphogenic codes" (Hogan, 1999), sets of species-specific rules that are expressed repeatedly and in harmony to develop the colonial landscape.

COLONIAL ARCHITECTURE: GENETICS VS. ENVIRONMENT

A fundamental requirement for understanding development is an appreciation of the relationships between genes and morphologies (Nijhout et al., 1986). Such relationships can

be elucidated experimentally when the growth patterns of different-structured isolated branches (Fig. 1) or nubbins (isolated fragments in the size of a single to few polyps; Shafir et al., 2001; Fig. 2) from *S. pistillata* colonies are followed over time, and modification of the developed phenotypes are quantitatively and theoretically analyzed. The above characterizations are probably genetically controlled. However, they are also clearly affected by environmental and a variety of biological challenges. Colony architecture is probably a character shaped by selection, so trade-offs between that trait and other traits may define a suite of morphological responses, some influenced by genetics, others by epigenetic impacts. In *S. pistillata*, a developing colony responds to perturbations by a set of rules that "canalize" (Waddington, 1942) growth pattern to the typical species morphology. This was first illuminated by Loya (1976), who demonstrated that in broken *S. pistillata* colonies, the lost spherical structure is regained by a differential growth pattern, involving fast growth in regenerating parts together with a reduction in growth in the intact branches. Photosynthetic products are channeled to the regenerating parts (Rinkevich and Weissman, 1987).

Even a single separated polyp (or several separated polyps) from a *S. pistillata* colony has the capacity to develop a new colony (Shafir et al., 2001; Fig. 2). Therefore, as implied from the findings presented previously, an individual colony may be regarded as a "whole", where intrinsic orders of branch growths and related physiological parameters (such as sharing of resources between different branches, simultaneous aging processes, and reproductive activities; Rinkevich and Loya, 1979, 1983, 1984, 1985b, 1986, 1987; Rinkevich and Weissman, 1987; Rinkevich, 1991; Rinkevich et al., 1991) "produce" the structure we are familiar with. It is suggested here that the genetic blueprint of a colonial organism is activated to ensure that the new branches added and the enlargement of existing branches will acquire the rules for the species-specific landscape. Scientists could further explore the importance of genetics for colony landscape by creating simulating programs that are able to orchestrate the

complex structure of a colony into mathematical language (Bell, 1986; Kaandorp and de Kluijver, 1992; Kaandorp and Sloot, 1997; Kaandorp, 1999), by application of architectural models to branching forms of colonies (Dauget, 1991a,b, 1994), by applying physical attributes to morphologies (Abelson et al., 1993) and by adopting other approaches for understanding morphologies (Blackstone and Yund, 1989; Buss and Blackstone, 1991; Blackstone and Buss, 1993; Cartwright et al., 1999).

A branching pattern develops by adding new components to an existing framework (Bell, 1986). In hermatypic branching forms, where past morphologies cannot be eliminated as the result of their calcium carbonate template, descriptions and analyses of dynamic branching processes along ontogenesis may reveal the rules of branching systems. Merely mimicking the rules for colony formation, by assumptions that create simulated colonies, is not enough, although in the past such approaches have provided insight into morphology. For example, Raup (1966) has shown that just four parameters were needed to simulate the gross form of a coiled shell and that actual specimens were not randomly distributed in the total spectrum of possible theoretical forms generated by computers. When simulating branch architecture, Bell (1986) distinguished between 3 different levels of biological reality: "blind", which is a simulated form oblivious to its environment; "sighted", where each step in the development is influenced by the environment but is unaware of its connectivity within the organism; and "self-regulatory" in which astogeny is controlled from within the organism using internal and possible external information. The "blind" aspect of branching architectures, in other words the genetic information, is the crucial level for construction. Some rigid forms may not accept the "sighted" or the "self-regulatory" aspects.

More than two decades of studies on different life history traits of *S. pistillata* have resulted in the accumulation of a vast amount of information regarding a variety of aspects that can be helpful when analyzing colony architecture. Flexibility and variations in colony landscape

formation may be controlled by specific genetic rules, as may the general spherical structure of the colony and the iterative processes of branching. Although there is yet no direct evidence for this, the possibility of phenotypic plasticity in a colonial organism, being a construct of genetic rules for morphologies (Zilberberg and Edmunds, 1999), should be seriously taken into consideration when studying landscapes of marine invertebrate branching forms. Such an approach may also enforce controversial views regarding the "origin" of colony landscape. Evolutionary biologists may foresee the characteristic "species-species" phenotype as the outcome of selection processes favoring phenotypic plasticity or environmental specialization (canalization), leading to a phenotype consistently conveys the highest fitness (Weinig, 2000). On the other hand, developmental biologists may favor the phenotypic expression as an adaptive character where both, genetics and environment, shape the organism's landscape. These ideas should be taken into account when dealing with colony formation and landscapes of branching colonial organisms.

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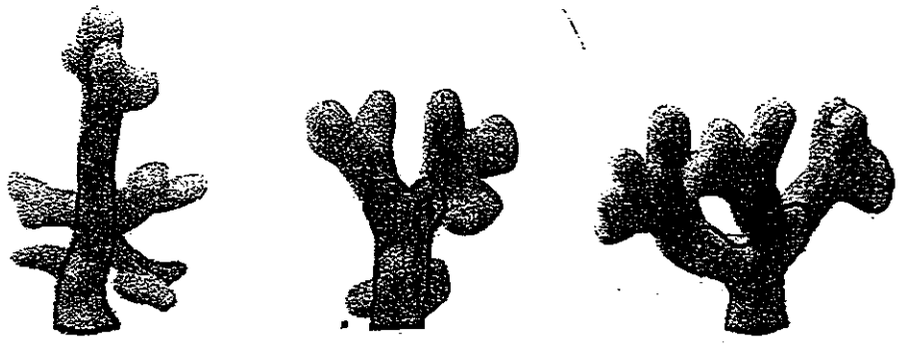

Fig. 1. Different pattern formation of colonial growth resulting from each initial fragment structure. Fragments a to c were taken from the same *S. pistillata* colony and represent the typical growth after 11 months of *in situ* culture. a = single tip branch that developed new lateral branches and relatively little apical growth; b = starting bifurcated branch that developed two ramified, well-defined sets of branches; c = ramified single branch that developed three well-defined sets of different branches. The initial sizes of the branches are confined within the black lines. Photographs taken by L. Shaish.

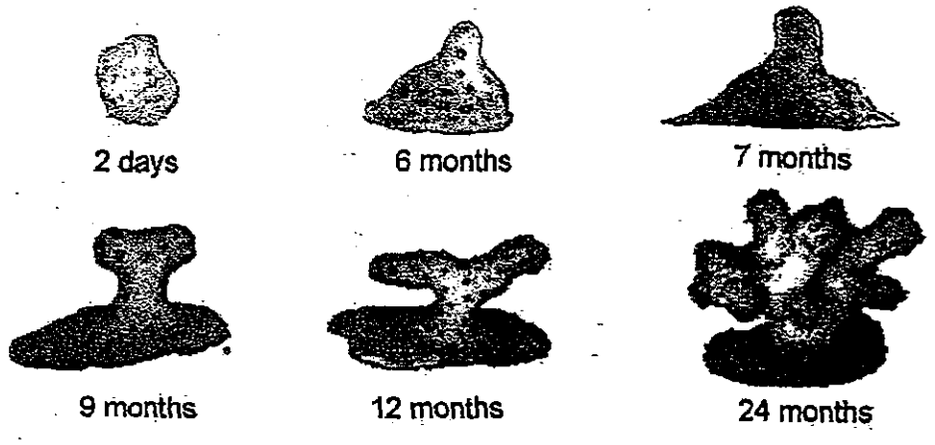

Fig. 2. From a nubbin, a structureless minute fragment of the branching coral *Stylophora*, to a whole, newly organized colony. Major steps in the well-organized establishment process of the species-specific architectural structure. These steps morphologically mimic the process involved in the astogeny of a *Stylophora* colony from settled and metamorphosed planula-larvae. Photographs taken by S. Shafir.

3. Colony pattern formation: studying the rules for massive coral forms.

INTRODUCTION

Bilaterally symmetrical body planes in contemporary metazoans are believed to have evolved from a radially symmetrical ancestor (Martindale & Henry 1998; Meinhardt 2002). Radial symmetry, where properties of two or more major planes of mirror symmetry characterize the animal's body plan, is confined in living groups to polyps of cnidarians and ctenophores (Goldstein & Freeman 1997; Martindale & Henry 1998). Their radial symmetry, however, is incomplete and in colonial forms does not embrace the entire colonial organization. Moreover, with regard to the polyp structure, most adult cnidarians possess indicators of bilateral symmetry showing changes and modifications to the stereotyped radial polyp pattern formation (Beklemishev 1969; Martindale & Henry 1998; Gershwin 1999). This raises the question of the evolutionary robustness of radial symmetry. In other words, are there pre-existing rules for fixed radial symmetry that "canalize" (Waddington 1942) different developmental processes? Or, is radial symmetry a spatio-temporal expression of phenotypic plasticity?

With the exclusion of short planktonic larval phases, reef building corals are sessile. Most are also colonial, three-dimensional tessellations of similar sized modular units, the polyps. A variety of colonial forms (i.e., branching, massive, encrusting) are formed by continuous iterative constructions of radiated accretive skeletogenesis, morphologically expressed as polyp budding. Colonial forms also have plastic architectures that allow corals to adjust to different environmental regimes (literature cited in Helmuth et al. 1997; Sebens et al. 1997; Muko et al. 2000). One of the most common coral genera found in reef communities is *Favia*, characterized by a massive colonial form packed with iterated units (the polyps). Corallites are plocoid (a type of colony formation where polyps have separate walls) and new polyps are formed by intratentacular division. *Favia* species have wide depth ranges on reefs, have wide

geographical ranges, are tolerant to a wide range of environmental conditions and represent high phenotypic plasticity (Veron 1986). Within a given *Favia* species, one colony may be morphologically different from colonies growing at other reef habitats or within the same habitat. Morphological variations include the parameter of polyp size (Veron 1986; Fig. 1). Here, the Red Sea coral *Favia favus* has been selected to further evaluate the morphological architectural rules of polyp budding in this species, especially those that are correlated with polyp size.

MATERIALS AND METHODS

For this study, 6 large *Favia favus* colonies (marked A to F; Fig. 1) were chosen randomly at ca 4 m depth in front of the Inter-University Institute at Eilat, Northern Red Sea. On each colony, 2-6 rectangles (6.1 X 9.0 cm, each, fitted Nikonos IV underwater camera extension macro tube frame, reproduction ratio 1:2), were permanently marked by thin stainless-steel nails. The number of rectangles correlates with colony sizes. Slide photographs were taken at intervals of ca 3 months for a period of 3 years. Within each frame 3 - 9 polyps were chosen for serial follow up morphometric measurements (total numbers of polyps: 190; Colony A = 27, B = 24, C = 39, D = 48, E = 18, F = 34). Slides were digitized and calculations were made for each polyp's surface area, perimeter and when applicable, on the process of budding by using an image analysis program (Tina 2.0). At the beginning the 6 colonies, with the exception of colonies C and D, varied significantly in average polyp size (polyp's surface area; Duncan's Multiple Range Test, $p<0.05$; colony A = 1.77 ± 0.28 cm^2, B = 2.25 ± 0.38, C = 0.96 ± 0.07, D = 1.02 ± 0.14, E = 0.63 ± 0.06, F = 2.06 ± 0.08, mean±s.d.; Fig. 1).

RESULTS AND DISCUSSION

We followed 190 *Favia* polyps during a period of 3 years (1121 days) in which 78 polyps (41.1%) underwent intratentacular budding. The budding operation was surprisingly slow; underestimated as 12.5 months on the average (colony A = 6.9 ± 2.2 months, n = 8; colony B

= 10.2 ± 5[12]; C = 18.4 ± 9.9 [24]; D = 19.8 ± 5.3 [20]; E = 9.3 ± 3.6 [7]; F = 10.3 ± 3.7 [7], mean±s.d.; Fig. 1). In 41 polyps, the budding process either began before the first set of photographs were undertaken, or was not completed at the end of the research. In 37 polyps (19.5%) the whole budding process was initiated and terminated within the three-year time frame of the study. Data on these dividing polyps is analyzed below. The first sign for polyp budding was the appearance of an additional mouth in the center of the polyp. The calcified wall that separated both mouths was formed later on, sometimes up to two years from the date when the second mouth was first recorded. Since observations were taken every 3 months, we are unable to provide an unbroken record of this process, which has the properties of a continuous phenomenon (in preparation). Budding was documented in polyps of different sizes, small and large alike, at different sites along the colony surface area contour, even within a single colony. Superficially, polyp budding in *Favia favus* is a highly variable trait.

To further analyze the rules that govern budding in *Favia*, we adapted the concept of canalization (Waddington 1942) that represents the reduced expression of variation at the level of the phenotype (Kawecki 2000). Most studies of canalization have actually involved monitoring the response of a trait to some type of perturbation (Gibson & Wagner 2000). Polyp budding in *Favia*, seems to be a response to spacing the development of "distorted" polyps on a sheet of tissue, where attached polyps are continuously growing (see Fig. 1). We develop, therefore, a scenario for polyp budding which considers the importance of two morpho-metric covert developmental fields (see below) based on the view that radial symmetry, the ancestral body plan of the cnidarians (Beklemishev 1969; Martindale & Henry 1998), is a decisive facet in shaping the polyp's morphology. According to this scenario, two stereotypic radial fields of the polyp, not its size or age, are the developmental constraint that provides signals for polyp budding. A typical polyp in *Favia* does not possess the radial symmetry prototype (Fig. 1). In a *Favia* colony, each corallite projects slightly above the

colony surface and has its own wall. Corallites are not circular, usually forming polygonal structures with 4-7 differently sized side walls that are shaped in accordance with the spacing of neighboring polyps. Such variations in corallite size and morphologies, as well as the apparent lack of any measurable trigger for polyp budding (pers. observ.), may give the impression that budding is poorly regulated at colony as well as at polyp levels.

However, we found that with respect to the stereotypic radial symmetry of the polyps, two simple corresponding morphometric fields may provide the basic cues for bud initiation (an example is illustrated in fig. 2): the first is the hypothetical circle (with radius r_p) calculated by the transformation of the polyp's real perimeter (P) to the equivalent perfect radial-symmetry circle of $P=2\pi r_p$. The second is another hypothetical circle (with a different radius, r_a) calculated by the transformation of the polyp's real surface area (A) to the equivalent perfect radial symmetric circle of $A=\pi r_a^2$ (shaded circle in fig. 2). The ratio of the two radii, $R = r_p/r_a$, greater than, or equal to the average value of 1.14, has been found in 33 (89.2%) of the cases (in all 6 colonies, regardless of their polyp size) before intratentacular budding began. The average R for the other 4 diving polyps was 1.198±0.009. After budding, R values dropped to 1.10 on the average. Polyps that did not divide during the 3-year observation period (n=112), had an average R = 1.09. In a perfectly circular-shaped polyp, this ratio values 1.0, and a shift from this ideal morphometric value may thus signal the diversion from radial symmetry. Thus, polyp formation in the genus *Favia* features a quantitative trait which may represent genetic canalization (Waddington 1942; Kawecki 2000). We use the term "genetic canalization" to refer to the buffering of developmental pathways under conditions that are likely to be highly restricted (Gibson & Wagner 2000). Different polyps, in different *Favia* colonies, do not need to be the same size or age to start the budding process, even in colonies that differ significantly in their average polyp size. Thus the fixed threshold ratio for polyp spatial formation in *Favia* is a "size-free" morphometric value.

This is the first time that such a morphological threshold for bud formation is revealed in the cnidarians. In some colonial hydroids, spacing in the periodic pattern of polyp formation is established by a simple inhibitory field (Plickert et al. 1987). In other colonial hydroids, a simple shape metric, perimeter area divided by area $^{(0.5)}$ correlates with stolonal mat structures (Blackstone & Buss 1991). There is, however, no detailed study yet referring to the symmetrical properties of polyps in relation to budding initiation. It should also be stated that the evolved geometric relationship in polyp budding does not prove that the "decision" to bud is directly determined by this ratio.

The precise connection between the value R and polyp budding in *Favia* may be difficult to establish at this stage. However, whatever mechanisms are involved, the results of the present study may illuminate possible morphometric relationships within the cnidarian body plan constrains. It is argued (Rice 1998; Gibson & Wagner 2000) that canalization may increase the potential for evolutionary divergence since in canalysed processes selection does not "see" new genetic variations, which allow the accumulation of genetic differences. Pre-existing co-opted plasticity, after its admixure with new morphometric traits and the breakdown of the prevailing canalizing system are probably the triggers for developing of variable morphologies where tangential elements that were added along the growth plan changed animals axis of development.

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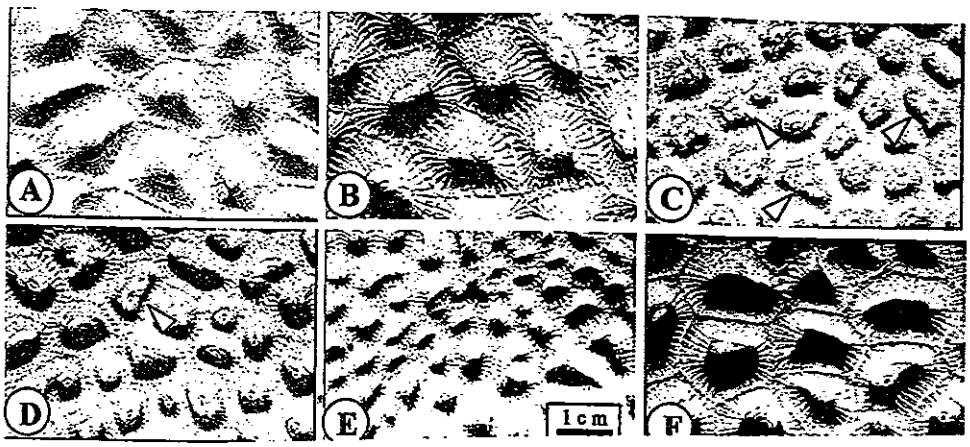
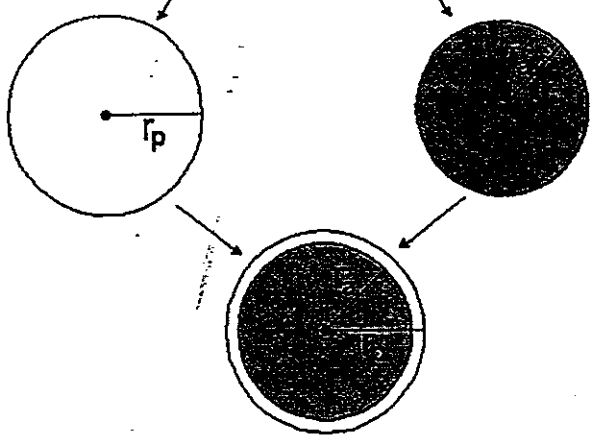


Fig 1

Fig 2

polyp's perimeter = 36.5 mm polyp's area = 86.1 mm²



$$R = \frac{r_p}{r_a} = \frac{5.81\ mm}{5.23\ mm} = 1.11$$

4. Impacts of particulated materials released from coral fish farms.

INTRODUCTION

Nutrient enrichment (either particulate or dissolved) is considered one of the main causes for environmental decline in oligotrophic waters harbouring coral reefs (Smith et al. 1981, Pastorok & Bilyard 1985, Wittenberg & Hunte 1992, Hunter & Evans 1995, Lapointe 1997, Roberts et al. 2002; and literature therein). High levels of nutrients stimulate macroalgal growth (Lapointe 1997) enabling them to rapidly cover living coral tissues. High nutrient loads also lead to an increase in phytoplankton biomass, resulting in turbidity and reduced submarine light levels which affect zooxanthellae photosynthesis (literature cited in Naim 1993, Genin et al. 1995, Dubinsky & Stambler 1996, Koop et al. 2001). Additionally, fast growth of filter-feeding invertebrates such as bryozoans, sponges and tunicates may out-compete corals for substrata resulting in a corresponding decrease in the diversity and abundance of hermatypic coral.

Many of the direct impacts of nutrient enrichment on the biology of corals are less clear, mainly because the above indirect effects may obscure the coral's physiological and biochemical response. Laboratory analyses indicated that nutrient enrichment leads to increased zooxanthellae abundance and chlorophyll *a* content in coral tissue (Hoegh-Guldberg & Smith 1989, Stambler et al. 1991 Stambler et al. 1994, Muller-Parker et al. 1994, Snidvongs & Kinzie 1994) and reduced coral growth and calcification rate (Kinsey & Davies 1979, Tomascik & Sander 1985, Davies 1990, Tomascik 1990, Stambler et al. 1991, Stimson 1992, Marubini & Atkinson 1999). Koop et al. (2001) found that the outcome of nutrient enrichment (N and P) depends on nutrient dose levels and on the coral species studied. An assessment of the variables used to evaluate coral health suggests that reproductive success (Tomascik & Sander 1987, Harrison & Ward 2001, Koop et al. 2001), growth and calcification rates (Hoegh-Guldberg et al. 1997, Steven & Broadbent 1997, Marubini &

Atkinson 1999, Ferrier-Pagès et al. 2000) are sensitive indicators of nutrient enrichment impacts. However, the scientific literature on nutrient enrichments includes contradictory reports regarding the effects of nutrients on hermatypic corals. For example, Atkinson et al. (1995) recorded high growth rates among 57 species of corals growing in high-nutrient seawater of the Waikiki Aquarium, Hawaii. Similar results were reported in several nutrient enrichment experiments carried out on corals in their natural environment (Gregg 1995, Hoegh-Guldberg et al. 1997, Steven & Broadbent 1997). Meyer & Shultz (1985 b) and Lieberman et al. (1995) documented enhancements in skeletal deposition, tissue growth rates and reproductive efforts in branching corals harbouring schooling fish that provide a continuous supply of nutrients to the corals via fish excreta.

Intensive net pen fish farming, a rapidly expanding industry throughout many of the world's coastal regions, is known to release large quantities of dissolved and particulate matter to the surrounding waters (Brown et al. 1987, Beveridge 1996, Karakassis 2001). Pitta et al. (1999) showed that warm water fish farms discharge dissolved and particulate nutrients to the surrounding environment throughout the year. If we accept the view that nutrient enrichment is detrimental to coral health, the recruitment, growth and survival rate of corals in the vicinity of fish farms should be inferior to those of corals in oligotrophic waters. The rapid and continuous deterioration of the coral reef in Eilat (Epstein et al. 1999, 2001) and the nutrient and particle loads generated by the fish farms (Angel et al. 1998) evoke increasing concern (Mancy 1993) and debate regarding the environmental impacts of cage aquaculture on the coral reef. Despite these concerns, detailed studies that critically evaluate this topic have not been undertaken.

In this study we have assessed the impacts of a commercial fish farm near Eilat, Israel on some life-history parameters of selected coral species. For this purpose, transplanted and resident coral colonies adjacent (10-50 meters) to the fish cages were compared to

transplanted and resident colonies situated at a nearby relatively clean (reference) site. Two branching coral species, *Acropora eurystoma* (Klunzinger 1879; in Wallace 1999) and *Stylophora pistillata,* commonly found in the coral reefs of Eilat (Loya 1972) were chosen. Survival rates, growth rates, reproductive activity and lipid storage levels were used as indicators of nutrient enrichment impacts.

Materials and Methods

Study Sites. The Ardag fish farm (29°32.45'N, 34°58.40'E) is situated at the north shore of the Gulf of Eilat (Aqaba), Red Sea (Fig 1) over a sandy seafloor. The net cages of this commercial farm (established in 1988) are stocked mainly with gilthead seabream (*Sparus aurata*). The farm consists of 3 parallel pontoons that support the fish cages. During 2001 the annual estimated discharge from the production in the central and western pontoons (both particulate and dissolved forms, calculated from Lupatsch and Kissil 1998) of the Ardag farm (see description of the experimental site, below) consisted of 92 tons nitrogen and 16 tons phosphorus.

The Ardag farm is located south of Nahal Arava, a dry riverbed that episodically (following winter rains) conveys large quantities of fine alluvial material to the Gulf. The amount of terrigenous unconsolidated sediment deposited in the area can suffocate or bury settled coral colonies. Thus, coral colonies are found in this region only on elevated hard substrata, mostly on artificial objects (ropes, concrete sinkers, automobile tires, etc.). In spite of the high sedimentation and the potential harmful effects of the fish cages, the presence of > 450 coral colonies representing at least 22 different genera was recorded during 2000 within a lateral distance of 30m from the fish cages (Angel et al. unpubl.). In 2001, the monthly average nutrient levels at the fish farm were 0.095 μM for nitrite, 0.385 μM for nitrate, 0.123 μM for orthophosphate and 1.016 μM for ammonia (David, Lazar and Post, unpubl.).

The coral reef in front of the Inter University Institute (IUI) of Eilat served as the reference site in this study. It is located 8 km southwest of the Ardag fish farm and adjacent to the Eilat coral reef nature reserve (Epstein et al. 1999). The reef has a low profile fringing structure, dominated by hermatypic coral colonies and associated invertebrates (Yahel et al. 1998). In 2001, the monthly average nutrient levels at the IUI site were 0.075 μM for nitrite, 0.264 μM for nitrate, 0.045 μM for orthophosphate and 0.057 μM for ammonia (David, Lazar and Post, unpubl.).

Particulate matter flux. The vertical flux of particulate matter was measured at the two study sites on 3 dates: August 20, 2001, October 22, 2001, and January 31, 2002. Sediment traps consisting of four PVC cylinders (5.6 cm diameter, 70 cm long) with collecting cups (Angel et al. 1995) were deployed at 6m depths for 48h on each of the sampling dates. Particulate samples collected in the cups were concentrated onto 47 mm diameter Whatman GF-C filters, rinsed with distilled water to eliminate salts, dried at 70°C for 24 h and weighed to determine dry weight (DW). The filters were then placed in a muffle furnace at 550°C for 8 h and subsequently weighed to determine ash-free dry weight (AFDW). The difference between DW and AFDW was attributed to combustible organic matter.

Coral growth. Ten colonies of *Acropora eurystoma* (ca. 15-20 cm diameter) were collected from the North beach of Eilat during February 2001 and stained for 24 h *in situ* with the vital stain Alizarin Red-S (10 mg/l) within transparent plastic bags. Corals were left in their natal site for a five day post-labeling period. Subsequently, 40 branches (ca. 3 cm each) were removed from each of the stained colonies (altogether 400 branches), split equally to form two groups of branches and attached in an upright, vertical orientation, to PVC plates by means of plastic clothes-pins. Four plates, each carrying 5 replicates of each coral colony (a total of 200 Alizarin stained branches), were moored adjacent to the western pontoon of the Ardag fish farm (6m from the fish cages) at 6m depths (seafloor depth at that site is 22m, i.e.,

we excluded possible impacts from resuspended bottom sediments). The plates with attached branches were situated between the western and central pontoons of the farm thereby ensuring maximal exposure to fish farm effluents, regardless of the direction of prevailing currents (Brenner et al. 2001). The second set of four plates (with 200 stained coral branches) was moored at 6m depths at the IUI site (seafloor depth at this site was 10-11 m). During the experiment, algae and encrusting invertebrates were removed from the PVC plates on monthly basis. The PVC plates were recovered in October 2001 (seven months later); branches were removed and examined to determine survivorship and growth at each site. Coral tissue was separated from the calcareous skeleton by 2 days immersion in a chlorine solution. Coral skeletons were dried, weighed (± 0.01 g accuracy), cut at the level of the Alizarin line with a side cutter to separate the post-labeling deposited skeleton and weighed again. Each coral branch was photographed with a scale before and after removal of the newly deposited skeleton. Two sets of photographs, one of the branch's lateral side and one of the upper surfaces were taken before and after skeleton removal. Photographs were digitized and analyzed with the image-analysis software TINA 2.07 to obtain height (h), width (w) and length (l) of each branch (Loya 1976). An "ecological volume" index was established for each branch by assuming a cylinder shape with volume $V = \pi r^2 \times h$, in which $r = l+w/4$ (Rinkevich & Loya 1983). This "ecological volume" represents the water volume occupied between the branches of the developing ramet.

In an additional set of experiments, coral nubbins (very small fragments of a colony, consisting of only a few polyps; Shafir et al. 2001) were taken during April 2001 from six *Stylophora pistillata* colonies, using a fine side-cutter. Nubbins were sampled from three colonies from the north beach of Eilat and three from the IUI site. The advantage of using nubbins is that a large number of genetically identical replicates can be obtained from a single coral branch, therefore causing minimal stress to the donor colony (Davies 1995, Shafir et al.

2001). The exposed skeleton surfaces of the freshly cut nubbins were dried and glued with a drop of cyanoacrylate glue (SuperGlue) to PVC plates. The plates were then transferred to a small platform moored at 6m depths. Trauma was minimized by limiting air exposure to a few minutes and by rinsing each nubbin with fresh seawater upon isolation. 120 nubbins were glued onto three PVC plates at each of the two experimental sites. Nubbins were photographed at the beginning of the experiments and after 3, 4 and 13 months. Photographs were analysed by the image analysis package TINA 2.07. Survival rates and the percentages of nubbins that spread (laterally on the plates) were recorded while nubbins growth rates were assessed from the lateral expansion of coral tissue, as typically reported for this species (Shafir et al. 2001).

Reproductive activities. In order to assess coral gonadal development, we chose 14 colonies of S. pistillata (> 10 cm diameter) growing naturally at 10-17 m depth on artificial substrata near the Ardag fish cages (< 50 m distance). Fourteen similar sized S. pistillata colonies, located at the same depth range were selected at the IUI control site. Single branch samples, representing the reproductive activity of the colonies (Rinkevich & Loya 1979), were taken from each colony in January, May and December 2001 (these months constitute two phases in the coral's reproductive cycle). Branches were transported to the laboratory in seawater plastic bags and were immersed in a 50% seawater-Bouin's fixative solution for a period of two weeks. Following this, a 5 mm tissue sample was removed from each of the decalcified branches and prepared for histological examination (Rinkevich & Loya 1979). The paraffin-embedded tissue was sectioned (5-6 μm thick sections), mounted on glass slides and stained with eosin and haematoxylin. For each sample, 8-10 polyps were examined in serial sections to determine presence of male gonads and numbers of female oocytes per polyp, stage of gametogenesis and oocyte sizes (sensu Ward & Harrison 2000).

Lipids in coral tissue. Three fragment replicates, 2-3 cm each, were sampled at the end of the coral growth experiment (7 months under local ambient conditions) from 8 randomly chosen ramets of *A. eurystoma* per site. In addition, fragments were sampled from 12 of the resident *S. pistillata* colonies that were studied for reproductive activities, at each of the study sites. Fragments were sampled randomly. All samples were placed in 10% formaldehyde-seawater solution for 24h, rinsed with distilled water and extracted in chloroform: methanol (2:1) for 24 hrs (Harriott 1993). The extracts were evaporated at 60°C and weighed to the nearest ± 0.0001g. The remaining coral branches were decalcified in 10% HCl, dried at 60°C, and weighed. Lipid content was calculated as the weight of lipid extract / (weight of lipid extract + tissue weight) × 100%. The lipid content of each colony was calculated as the average of three replicates.

Statistical analyses. All statistical tests were performed using SPSS 8.0 (SPSS Inc., 1989-1997) and SAS (SAS Inst. Inc., 1999) statistical packages. The assumption of normality and homogeneity of variance were tested by Kolmogorov-Smirnov and Levene's statistical test, respectively. Post hoc multiple comparisons of means were carried out by Tukey's HSD test. When required, data was ln transformed. The non-parametric Mann Whitney test was employed where results did not show a normal distribution pattern. Results are presented as average ± SD (standard deviation) except where indicated.

RESULTS

Particulate matter flux rates

Total particulate matter and particulate organic matter flux rates on the three sampling dates were 5, 6 and 2 fold higher and 10, 13 and 7 fold higher, respectively, at the fish farm site as compared to the IUI site (Table 1). The organic component of the particulate matter was >44% at the fish farm site whereas it was < 24% at IUI.

Survival and growth rates

The survival rates of *Acropora eurystoma* branches at both sites were 100% throughout the 7 months that branches were maintained on the plates. There was no significant difference in the initial branch size between the two sites (Duncan's multiple range test, $p > 0.05$). However, after 7 months the branches at the fish farm site had increased 3-fold in weight and linear extension and 4-fold in ecological volume as compared to the branches at the IUI site (two-way ANOVA, $p < 0.001$; Table 2). At the end of the experiment, branches growing at the fish farm had developed into small bushy-shape colonies with lateral branches as compared to the limited development among branches at the IUI site (Fig 2A). Despite the high variability among different colonies, both linear extensions of the branches and weight increases were significantly higher at the fish farm site ($p<0.001$ and $p<0.05$, respectively; nested ANOVA). These site-specific differences were consistent among all 10 coral genotypes (10 different colonies) that were the source of the living coral tissue in the experiment.

We concentrated on nubbin survival and nubbin lateral growth rates on substrata in our studies on nubbins. Lateral growth (spreading) of the *Stylophora pistillata* nubbins was observed 44 days after the beginning of the experiments. There was deposition and accumulation of particulate matter on the plates around the nubbins, occasionally even partially covering the nubbins. This phenomenon was more pronounced at the fish farm site. While nubbin survival rates at the two study sites, after three and four months, were not different, the number of nubbins that spread on substrata and their lateral growth rates were higher at the IUI site (Table 3; $p<0.001$, two-way ANOVA). However, the nubbins' average growth rate at the fish farm site increased with time and doubled between the third and the fourth month of the experiment, whereas it remains constant, but high, at IUI (Table 3). After 13 months the small colonies that had developed from the nubbins at the fish farm site were significantly larger than the nubbins at IUI (19.2 ± 6.1 mm [n=25] vertical extension and 16.3

± 4.6 mm [n=60], respectively, t-test, $p < 0.05$; Fig 2B). With time, the nubbins fused, eventually forming a continuous layer of coral tissue and skeleton on the substrate (Fig 2B).

Gonadal development

The reproductive status of *Stylophora pistillata* was monitored at the two studied sites at the beginning and peak of the 2001 reproductive season (January and May) and at the beginning of the 2002 reproductive season (December). In total, 701 polyps were examined via serial histological sections taken from 28 *S. pistillata* colonies (269 polyps in January 2001, 216 in May 2001 and 216 in December 2001). The number of colonies with female gonads was higher at the fish farm than at IUI in both January 2001 (93 % vs. 71 %) and May 2001 (100% vs. 67 %). In December 2001, all of the colonies at both sites developed ovaries. The oocytes in the *S. pistillata* colonies growing near the fish farm were twice as large as their counterparts at IUI (52 ± 27 μm diameter vs. 26 ± 23 μm, respectively) although the difference was not statistically significant. The average number of oocytes polyp^{-1} (Fig 3A) was significantly higher at the fish farm than at IUI, and varied significantly between the three sampling dates (two way-ANOVA, $p<0.01$ and $p < 0.05$, respectively). At the IUI site the second reproductive season was characterized by a significant higher oocytes polyp^{-1} than the first season (Tukey's HSD Post Hoc test). The proportion of colonies with developing male gonads in both January and May 2001 was higher at the fish farm than at IUI (93% vs. 78% and 83% vs. 33%, respectively), yet it was even higher though not significantly different at the two sites in December 2001. The percentage of polyps with developing testes was significantly higher at the fish farm than at IUI throughout 2001 (74% vs. 49%, two-way ANOVA, $p < 0.001$, Figure 3B). There was an increase in the proportion of polyps with ripe testes (containing developed spermatozoa) during 2001 at the IUI, from 7% to 33%, as compared to a steady high value of 53% at the fish farm. At both sites, the highest percentage

of gonads containing spermatozoa was recorded in the following reproductive season, 83.3% and 100%, respectively.

Lipid contents

There was no significant difference in the lipid contents of *A. eurystoma* branches at the two study sites following the 7 month *in situ* experiment (Mean ± SD: 42 % ± 9 at IUI vs. 38 % ± 5 at the fish farm, n= 8). However, the average lipid content in tissues of naturally growing *S. pistillata* colonies sampled at IUI was higher than at the fish farm (36 % ± 5 vs. 30 % ± 3, respectively; Wilcoxon signed rank test, n=12, $p < 0.05$).

Discussion

An abundant body of literature (reviewed in Beveridge 1996, Black 2001), as well as local recent studies (Angel et al. 1998; Katz et al. 2002; David, Lazar and Post, unpublished) have shown that intensive net pen mariculture releases substantial amounts of both, particulate and dissolved forms of nutrients to the surrounding waters. As higher levels of nutrients have often been reported to negatively affect coral health, it was intriguing to discover that both *Acropora eurystoma* branches and *Stylophora pistillata* nubbins grew faster at the more "eutrophic" fish farm, as compared to the oligotrophic IUI site (the results obtained here may not reflect however, longer term, cumulative impacts of eutrophication; literature cited in Dubinsky & Stambler 1996). The same was observed with the increase in "ecological volume" values of developing branches that reflect actual water volume occupied between the branches of each colony. Moreover, survival rates of coral branches and nubbins of both coral species were high and not significantly different between the two study sites. Although past studies have suggested a trade off between coral growth and reproductive effort (reviewed in Rinkevich 1996), the results of the present study do not support this tenet since not only growth but also reproductive activities of *S. pistillata* were higher at the fish farm than at IUI.

These results seem to contradict studies that documented detrimental impacts of eutrophication on corals. Ward & Harrison (2000) reported a significant drop in production of oocytes and planula larvae among *Acropora longicyatus* and *A. aspera* colonies subjected to nutrient enrichments in the ENCORE experiment (Great Barrier Reef). Similar results were reported by Tomascik & Sander (1987) for *Porites porites* in the Caribbean. Our results also indicate that while higher growth rates and reproductive efforts were recorded at the fish farm (both are energy demanding physiological parameters, Rinkevich 1996) the lipid contents in tissues of naturally grown *S. pistillata* colonies at the reference site (IUI) was significantly higher than at the enriched fish farm site. It is possible that higher physiological-biochemical levels are associated with environmental conditions at the fish farm.

This observation begs the question: Are the fish farm nutrient effluents used as an alternative or supplementary source of coral nutrition?

Although higher levels of nutrients are generally associated with detrimental impacts on coral growth and reproduction, Ferriér-Pages et al. (2000) have suggested that the outcome of nutrient enrichment actually depends on the chemical form and concentration of these nutrients. It is well known, for example, that corals readily take up ammonium (Muscatine & D'Elia 1978, Grover et al. 2002) and phosphorus (D'Elia 1977). In some cases, it was proposed that organic matter might provide corals with an additional food source in hyper-oligotrophic waters (Hoegh-Guldberg et al. 1997, Anthony 1999, Anthony & Fabricius 2000). Meyer & Shultz (1985 a, b) and Lieberman et al. (1995) suggested that fish living in close association with branching corals may enhance coral skeleton, tissue and reproductive growth by providing nutrients via fish excretion and feces.

A large *in situ* multidisciplinary study that was carried out over the course of two years at the Great Barrier Reef, Australia (ENCORE; Hoegh-Guldberg et al. 1997, Ward & Harrison 2000, Koop et al. 2001) did not reveal many of the detrimental effects on corals generally

expected from nutrient enrichments (such as increased mortality and reduced reproduction and growth rates of corals). Increased concentration of nutrients had a number of conflicting effects on the organisms (corals, other reef invertebrates and algae) living within the ENCORE patch reefs. Nutrient types and concentrations may therefore, dramatically influence the directionality of biological response in reef dwelling organisms. In coral reefs subjected to numerous detrimental impacts (e.g. the coral reef of Eilat, Epstein et al. 1999, 2001) inflicted by large numbers of swimmers, snorkelers and scuba divers; various forms of anthropogenic pollution, siltation and sand deposition, etc., it is difficult to separate and quantify the relative contribution of each impact to reef decline. Therefore, our results challenge the prevailing notion among the general public (http://ecesorg/articles/static/98679240033115.shtml) that nutrient effluents released from intensive net pen fish farms in the Gulf of Eilat is the major detrimental factor contributing to the degradation of the coral reefs along the Israeli coast.

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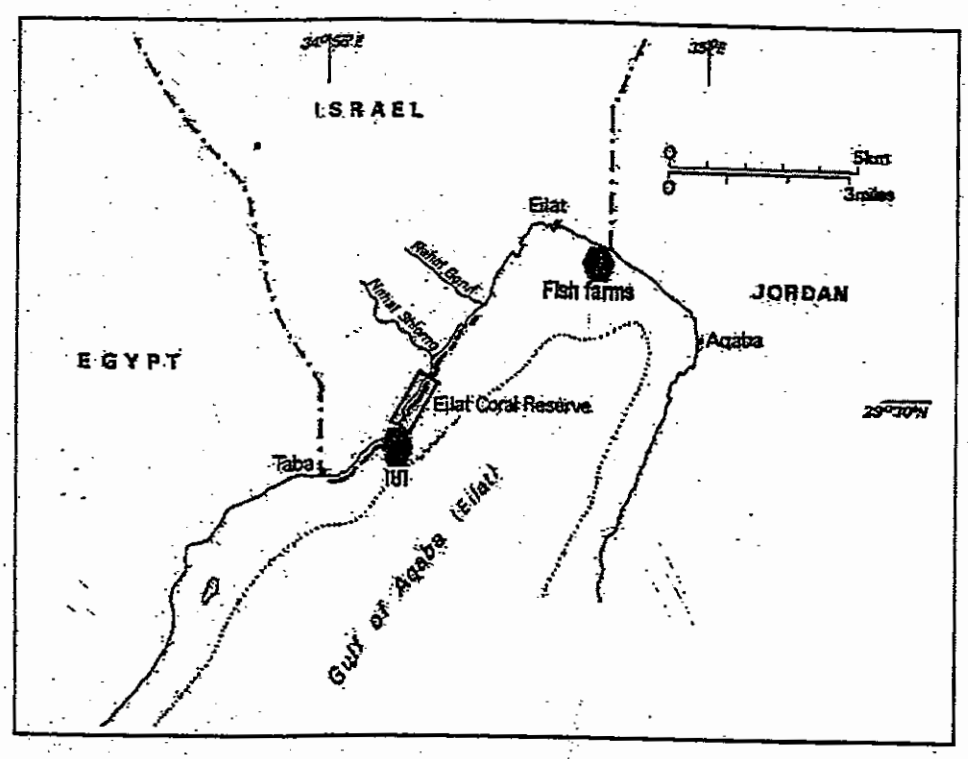

Fig 1

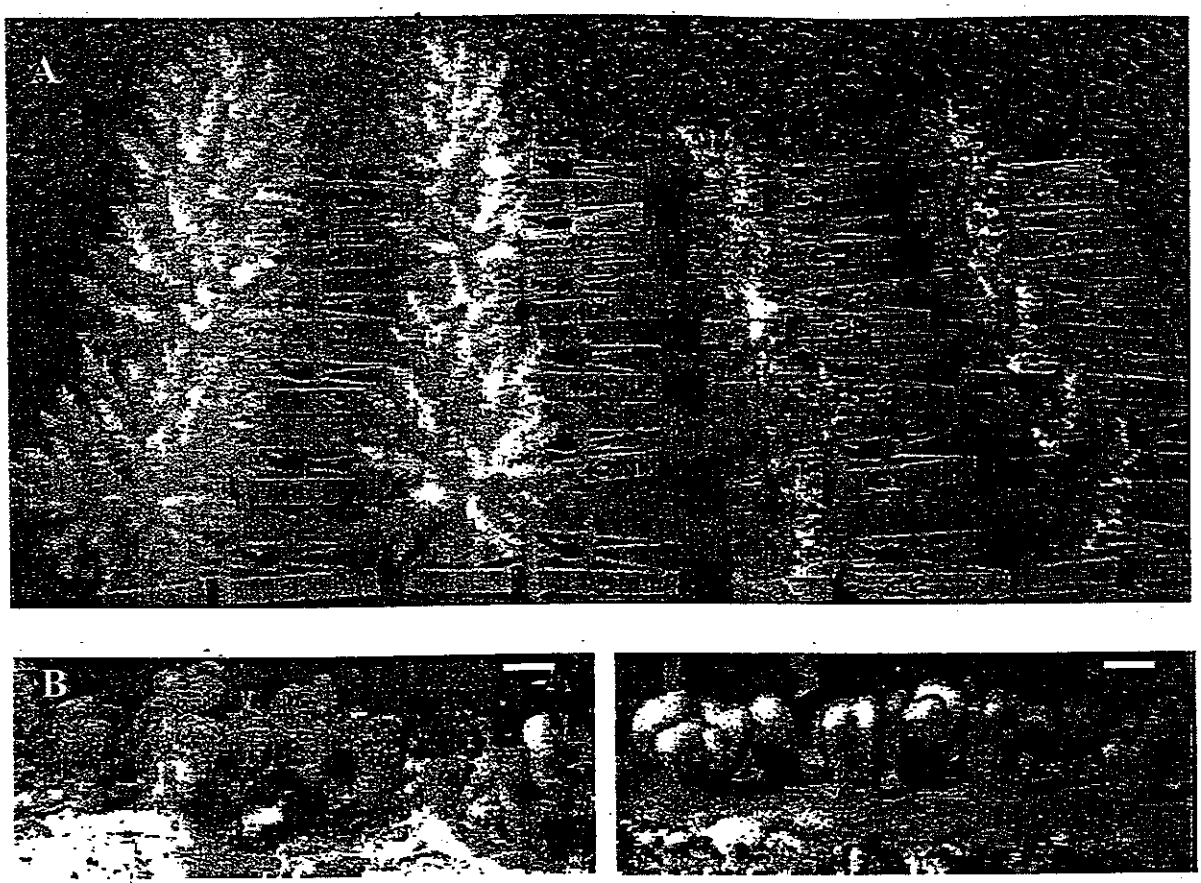

Fig 2

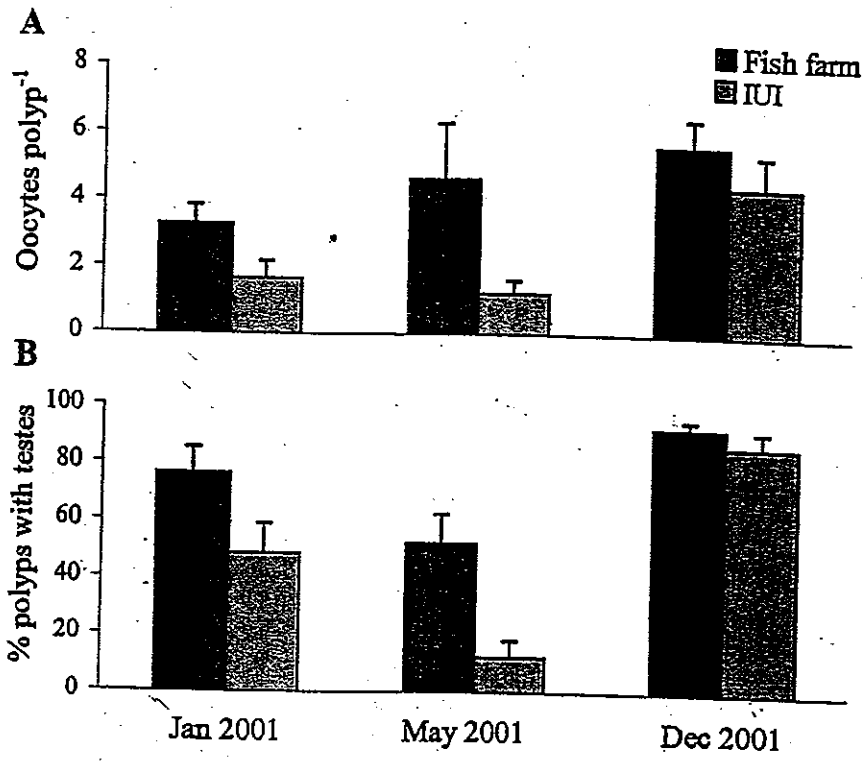
A
Oocytes polyp-1
8
6
4
2
0
Fish farm
IUI
B
% polyps with testes
100
80
60
40
20
0
Jan 2001
May 2001
Dec 2001

Fig 3

Table 1. Particulate matter (PM) and particulate organic matter (POM) flux rates (mean ± standard deviation) at 6m depth at the Ardag fish farm and at the Inter University Institute (IUI) sites. Results of Mann-Whitney test are indicated as follow: *p< 0.05, ns =not significant.

Date	Study site	PM flux rate ($g\ m^{-2}\ d^{-1}$)	Mann-Whitney results	POM flux rate ($g\ m^{-2}\ d^{-1}$)	Mann-Whitney results	Organic Component (%)
Aug 01	Fish farm	8.4 ± 1.3		4.1 ± 0.5		48.8
	IUI	1.7 ± 0.1	*	0.4 ± 0.1	*	23.5
Oct 01	Fish farm	8.8 ± 2.5		3.9 ± 0.9		44.3
	IUI	1.5 ± 0.7	*	0.3 ± 0.0	*	20.0
Jan 02	Fish farm	5.6 ± 1.5		2.7 ± 0.6		48.2
	IUI	2.9 ± 1.4	ns	0.4 ± 0.1	*	13.8

Table 2. Criteria for the estimation of *Acropora eurystoma* branch growth rates at the fish farm and IUI sites after 7 months growth on plates in the sea.

Criterion tested	Fish farm	IUI
Weight increase (g)	10.4 ± 6.1	3.8 ± 2.8
Linear extension increase (mm)	30.8 ± 8.7	9.6 ± 5.3
Ecological volume increase (cm^3)	211 ± 160	49 ± 45

Table 3. Survival, spreading and lateral growth (LG) rates of *Stylophora pistillata* nubbins after 90 and 120 days. Differences between the sites in terms of percentage of survival rates and percentage of nubbins spread, i.e., nubbins growing laterally on the plates, were analyzed by the Wilcoxon signed rank test whereas lateral growth rate was tested using 3-way ANOVA. Results of the statistical tests are presented as follows: * $p < 0.05$; ** $p < 0.001$; ns = not significant.

cation	Nubbins	After 90 days			After 120 days		
	(n)	n (% survived)	% spread	LG ($mm^2 d^{-1}$)	n (% survived)	% spread	LG ($mm^2 d^{-1}$)
sh farm	189	177 (94%)	66	0.50 ± 0.41	170 (90%)	82	1.03 ± 0.90
I	195	192 (98%)	87	1.41 ± 1.06	179 (92%)	92	1.72 ± 1.52
at. Test		ns	*	**	ns	*	**

5. Impacts of particulate matter on nubbins.

Introduction

Although eutrophication is generally considered one of the main causes for the coral reef decline (Wittenberg and Hunte, 1992; Hunter and Evans, 1995; Roberts *et al.*, 2002) it is not yet clear which one of its associated agents (nutrients, sedimentation, etc.) is the most detrimental (Koop *et al.*, 2001 and literature therein). Elevated nutrient concentrations have been reported to reduce coral growth and calcification (Tomascik, 1990, Stambler *et al.*, 1991; Stimson, 1992) and negatively affect coral reproduction (Harrison and Ward, 2001; Koop *et al.*, 2001). High levels of suspended sediment have also been demonstrated to negatively affect coral larvae, juveniles and adult colonies (Wittenberg & Hunte, 1992; van Katwijk et al., 1993; Gilmour, 1999; Torres, 2001). However, enhanced skeletal and tissue growth rates have been observed in branching corals supplied with dissolved and particulate waste released by inhabiting schooling fish (Meyer & Shultz 1985a,b; Liberman *et al.*, 1995). Antony & Fabricius (2000) further showed shifting roles for heterotrophy and autotrophy in coral energetic patterns under varying turbidity conditions.

During the last decade, the fast development of intensive net pen fish farming in the northern part of the Gulf of Eilat, Red Sea, has evoked increasing concern regarding its eutrophication impacts on local coral reefs. *In situ* fish farms continuously release particulate and dissolved nutrients to the surrounding environment throughout the year, affecting water quality and increasing turbidity (Brown *et al.*, 1987; Angel *et al.*, 1995; Pitta *et al.*, 1999; Karakassis, 2001). Particulate organic matters as well as dissolved flux rates near the fish farm cages at Eilat are indeed several folds higher than in distant reefs (Lupatsh and Kissil, 1998; Bongiorni *et al.*, in press). In order to test the impacts of nutrient enrichment in the

vicinity of Eilat's fish farms on corals, we (Bongiorni *et al.*, in press) carried several field experiments during 2001. During the first field experiments, branches of common acroporid species *Acropora eurystoma* were transplanted adjacent to fish farms and in a control reef situated 8 km southern. After seven months of *in situ* maintenance, branches at the fish farm, surprisingly grew significantly faster than in the control reef, while survivorship in both sites was 100%. During the second experiment, nubbins (Shafir *et al.*, 2001) of another common branching form, *Stylophora pistillata*, secured at 6 m depths at both sites showed no significant difference in survivorship but revealed significantly higher lateral growth rates at the control site as compared to the fish farm (a 3-months observation). After 13 months of *in situ* mariculture, however, the nubbin's linear growth (height) at the fish farm was higher than at the control site. The major question that emerged was: is the reduced lateral spreading rate of nubbins near the fish farm due to their partial coverage by particulate matter or by the higher dissolved nutrient load?

We describe here a follow-up field experiment in which nubbins of *Stylophora pistillata* were moored onto small PVC platforms in three different spatial orientations: the upward position, facing water surface; the vertical position, orthogonally to seafloor; and the downward position, facing the sea floor.

Materials and Methods

Study sites

The Ardag Fish Farm, the site of the experiment, is situated over a sandy sea floor at the northern shore of the Gulf of Eilat, Red Sea (29°32.45'N, 34°58.40'E). The farm maricultures gilthead seabream (*Sparus aurata*). At the time of this study, the estimated discharge from the fish cages was 92 tons nitrogen and 16 tons phosphorous (Lupatsch and

Kissil, 1998). The control site, located in front of the Interuniversity Institute (IUI) at Eilat (8 km southwest), is characterized by a low profile fringing reef, dominated by hermatypic coral colonies and associated invertebrates (Yahel *et al.*, 1998). In 2001, nutrient levels at the fish farm ranged between 0.003-0.277 μM nitrite, 0.065-1.058 μM nitrate, 0.028-0.44 μM phosphate and 0.089-2.5 μM ammonia while at the IUI site ranged from 0.001-0.325 μM for nitrite, 0.03-1.204 μM nitrate, 0.018-0.075 μM phosphate and from 0 to 0.14 μM ammonia (B. Lazar, A. Post, E.David, unpubl.). Average particulate matter flux rate measured in August and October 2001 were 8.5± 2.15 g m^{-2} d^{-1} at the fish farm and 1.59 ± 0.55 g m^{-2} d^{-1} at the IUI site with organic content reaching 47% and 24%, respectively (Bongiorni *et al.*, in press).

Experimental set-up and analysis

In August 2001, six branches were harvested from six different *Stylophora pistillata* colonies, of which three grew at the northern part of Eilat's coast and three at the control site (IUI). Nubbins (the average surface area 31.1 ± 9.7 mm^2, approximately 4 polyps each) were pruned from the above branches using an electrician's wire cutter and immediately immersed in seawater in order to minimize stress conditions (Shafir *et al.*, 2001). The exposed skeleton surfaces of the freshly cut nubbins were dried and glued with a drop of cyanoacrylate glue (Super Glue 3, Loctite, Ireland) to the three different sides of a U-shaped PVC bar. This method enables us, once *in situ*, to orientate the nubbins either facing the seawater surface (the upward position), facing orthogonally to the seafloor (the side position) or facing the seafloor (the downward position). Three PVC bars were fixed on each site to small platforms moored at 6m depth, and at least 5 m above the sea floor in order to avoid possible effects due to sediment resuspension. A total of 765 nubbins (270 in upward positions, 239 in side positions, and 256 in downward positions) were moored in the fish farm at a 6 m distance from two adjacent pontoons. At the IUI site, 782 nubbins (270 in upward positions, 241 in side positions, and 271 in downward positions) were moored. The nubbins were photographed at

the beginning of the experiments and after 50 days to follow lateral growths on substrates (Shafir *et al.*, 2001). Thereafter, isogeneic tissue-to-tissue contacts with neighbouring nubbins that developed from lateral extension resulted in morphological fusions. Nubbin survivorship and the percentage of nubbin that spread laterally on substrates were directly recorded while nubbins' growth rates were calculated from the *in situ* photographs, using the image analysis package TINA 2.07.

All statistical tests were performed using SPSS 8.0 (SPSS Inc., 1989-1997) and SAS (SAS Inst. Inc., 1999) statistical packages. The assumption of normality and homogeneity of variance were tested by Kolmogorov-Smirnov and Levene's statistical test, respectively. Post Hoc multiple comparisons of means were carried out by Tukey's HSD test. Results are presented as average ± SD (standard deviation).

Results

Survival and spreading percentage

All nubbins pruned from one of the colonies died in both sites within the first month and were excluded from the calculations. This left us with 700 nubbins at the fish farm (245 in upward positions, 205 in side positions, 250 in downward positions) plus 622 nubbins at the IUI (227 in upward positions, 178 side positions, 217 in downward positions). Within 15-20 days, nubbins started to regenerate tissue over the exposed skeletal parts and in contact zones with the substrate which enabled them to strongly attach to the PVC surfaces. Thereafter, they started to grow laterally. During this period and thereafter, we observed the accumulation of sediment and organic particles on nubbins and between them. At the end of the experiment (Day 50) a thick layer of settled material was formed to cover the lateral growths the between nubbins and in some cases even the whole nubbins. This phenomenon was distinctively recorded at the fish farm site especially on the upward positioned nubbins. At the IUI the

sediment load was minimal and did not cover all the nubbins. At the fish farm, a variety of encrusting algae and invertebrates (mainly sponges and bryozoans) settled on the downward position PVC surfaces (Fig 1), a phenomenon not yet seen at the IUI. Nubbin survival was 94% at the fish farm and 93% at the IUI (Wilcoxon signed rank test, $p > 0.05$) and no significant differences were found as well in survival of nubbins between the three spatial orientations at each site (Wilcoxon test, $p > 0.05$). After 50 days, 66% of the nubbins that survived at the fish farm and 85 % at the IUI had spread laterally over the PVC surface. A significant difference in the number of nubbins that spread laterally was found only between the two downward position sets (Wilcoxon $p<0.05$, 51% vs. 92% at fish farm and the IUI, respectively). No significant difference was found between numbers of laterally spread nubbins on the side and upward positions (77 vs. 82% and 73 vs. 80% at the fish farm and the IUI, respectively; Table 1).

Nubbins growth

After 50 days, the three-way ANOVA performed for the lateral growths revealed significant impact for sites (fish farm vs. IUI), colony genotypes and nubbins orientations. At the fish farm lateral growth rates of downward and upward positioned nubbins was statistically lower than that of the side positioned nubbins (0.84 ± 0.68, 0.93 ± 0.66 mm^2 day^{-1} compared to 2.41 ± 1.41 mm^2 day^{-1}, respectively, two-way ANOVA, $p < 0.001$, post-hoc Tukey HSD test). At the IUI, growth of upward positioned nubbins ($1.74 \pm$ mm^2 day^{-1}) was statistically lower than nubbins oriented in downward and side positions (2.31 ± 1.44 and 2.17 ± 1.16 mm^2 day^{-1}, respectively, two-way ANOVA, $p < 0.001$, post-hoc Tukey HSD test). Analysis for side position growth revealed a faster growth rate at the fish farm than at the IUI (two-way ANOVA, $p < 0.01$, post-hoc Tukey HSD), while for the other two position sets, the growth rate at the IUI was significantly faster than at the fish farm (two-way ANOVA, $p < 0.001$, post-hoc Tukey HSD, Fig 2, Table 2). The growth rates of a specific colony genotype

varied significantly when testing was carried out between the different orientations. For example, at the IUI, growth of colony 1 nubbins in downward positions was significantly the highest for all 5 genotypes, the highest for the side positions, but among the lowest for the upward positions (Fig. 2). The growth rate of colony 3 nubbins at the fish farm was the highest for all 5 genotypes in the side and upward positions, but among the lowest for the downward positions. The growth rate of colony 4 nubbins, however, was intermediate as compared to the other *Stylophora* genotypes studied for the 3 positions tested in IUI and fish farm sites alike (Fig. 2).

Discussion

Several authors (Davies, 1990, 1995; Ferrier Pagés *et al.*, 2000; Shafir *et al.*, 2001; Koop *et al.*, 2001; Bongiorni *et al.*, in press) have already used coral nubbins as a sensitive tool for ecotoxicological and environmental studies. Davies (1995) also pointed out the resemblance of nubbins to small colonies of their respective species. Adopting this approach, small nubbins of a few polyps only, each taken from *Stylophora pistillata* colonies, served as a tool to test physical impacts of the organically rich particulate matter released from fish cages.

In both study sites, high numbers of nubbins survived and spread. The only difference found was the lower number of nubbins that spread in the downward position at the fish farm. Since particulate flux disturbance is not a problem at this orientation, decreasing the growth of nubbins in this position could have been induced by reduced light availability. Nubbins from *Stylophora pistillata* have already been found to respond to different light regimes, changing their growth patterns accordingly (Shafir *et al.*, 2001). At the fish farm, the higher flux of particulate matter in the water indirectly resulted in reduced light penetration (unpubl. observ.). Dim conditions at the downward oriented position also led to successful

development of encrusting algae and various invertebrates (recorded only at the fish farm) which further out compete the developed nubbins.

We (Bongiorni *et al.*, in press) also recorded lower lateral growth rates of upward position nubbins at the fish farm as compared to the IUI. This outcome most likely resulted from the higher amount of particulate matter released from the fish net pens that settled on the PVC platforms, burring lateral growths and partially covering the nubbins. Mechanical stress due to the high sediment load is considered among the main causes for low settlement and survival rates (Hodgson, 1990; Tomascik, 1991; Gilmour 1999). Our results on small coral fragments suggest that at the fish farm high particulate flux could represent the principal detrimental effect for new settled coral larvae and juvenile stages.

Interestingly, the high sediment load did not affect the "side situated" nubbins that grew even faster in the vicinity of fish farm than in the control site, probably responding to enriched nutrient levels in seawater (Bongiorni *et al.*, in press). These results furthermore suggest that enriched conditions, such those occurring at the Eilat' fish farm, may constitute an additional food source for corals.

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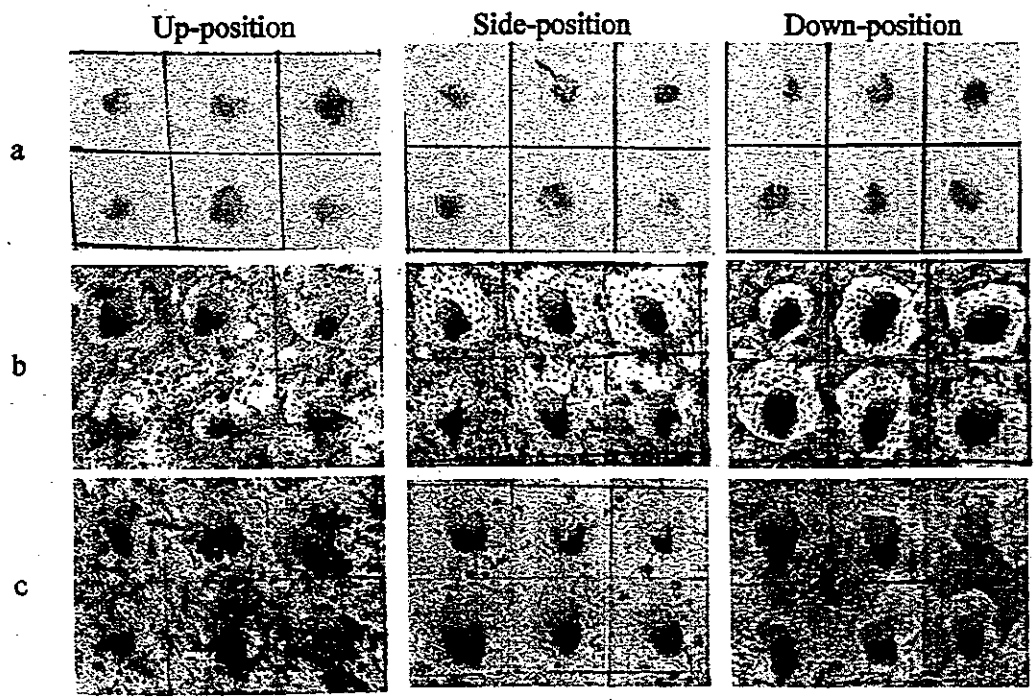

Fig 1

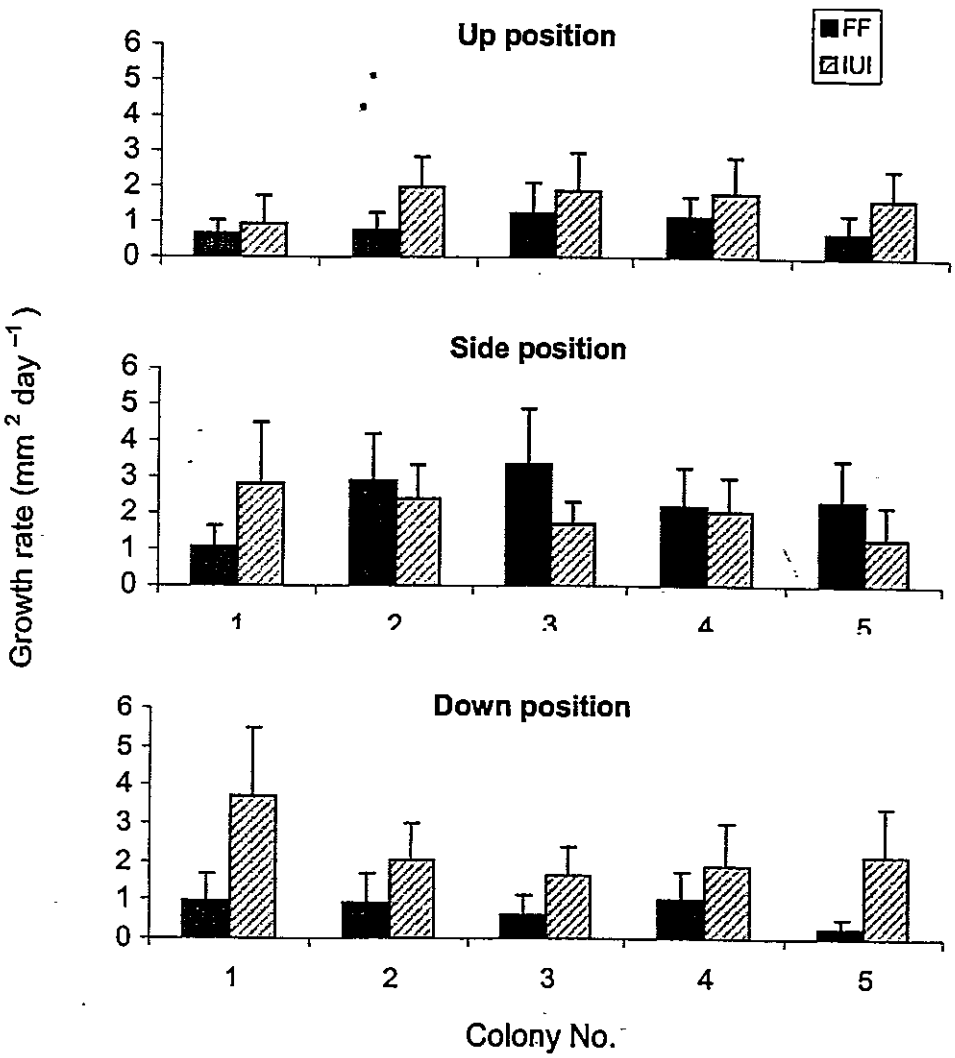
Up position
FF
IUI
Side position
Down position
Growth rate (mm 2 day −1)
Colony No.
0
1
2
3
4
5
6

Fig 2

Table 1: Nubbins survival and spreading percentages at different orientations in the fish farm (FF) and control site (IUI).

Colony No., location	Upward position			Side position			Downward position		
	n	% survival	% spread	n	% survival	% spread	n	% survival	% spread
1 FF	50	54	93	43	93	68	52	88	57
2 FF	51	90	78	42	100	74	49	100	59
3 FF	41	100	83	38	100	95	51	96	59
4 FF	50	100	64	42	86	78	52	98	57
5 FF	53	96	71	40	100	73	46	87	20
1 IUI	46	46	86	32	91	72	36	94	100
2 IUI	46	100	67	43	100	88	50	100	98
3 IUI	37	89	94	46	78	69	48	98	74
4 IUI	49	100	88	16	100	100	41	98	100
5 IUI	49	84	88	41	98	88	42	100	90

Table 2: Two-way ANOVA performed on growth rate of nubbins set up in the upward, side and downward positional orientations at both study sites (fish farm and IUI).

Source of variation	Upward position				Side position				Downward position			
	df	MS	*F*	*p*	*df*	MS	*F*	*p*	*df*	MS	*F*	*p*
Site	1	25.14	35.94	<0.0001	1	9.25	6.71	0.0099	1	382.24	390.80	<0.0001
Colony	4	7.76	11.09	<0.0001	4	10.33	7.50	<0.0001	4	22.31	22.81	<0.0001
Site × Colony	4	6.98	9.97	<0.0001	4	28.37	20.58	<0.0001	4	11.26	11.51	<0.0001

6. Tumors in corals from Costa Rica

Introduction

It is well documented that environmentally induced phenotypic plasticity in hermatypic corals can produce a wide variety of skeletal morphologies (Bruno and Edmunds 1997) including changes in the 3-D morphological architecture of colonies (Rinkevich 2002). Not all phenotypic plasticity forms are normal. Extravagant growths and sometimes a chaotic development of polyps in some coral species resemble in many ways the abnormal growth in higher animals, a phenomenon referred to as tumors (Squires 1965a,b, Lauckner 1980). A tumor in corals is a widespread phenomenon that has been reported in many species from at least 10 different families (Peters et al. 1986).

A tumor or a neoplasia is defined as a pathological process due to disruption of normal genetic control of cellular proliferation, differentiation and/or cell death, resulting in excessive and inappropriate growth of a cellular population which is genetically unstable, and which may progress through accumulation of further genetic alterations to become an invasive and metastatic form. It usually presents an excessive cellular proliferation of an inappropriate nature, unrelated to functional demand.

The diagnosis of neoplasia in the vertebrates is a relatively straightforward and standardized procedure and the parameters are simple to identify. This is not the case in more primitive animals such as in members of the phylum Cnidaria. In these organisms, tumor-like formations are easily observed macroscopically from the early stages of their formation; however, no microscopic differences were found when cytological comparisons were made between tumor and healthy coral tissues (Peters et al. 1986), even in cases where coral tumors overgrew large portions of colonies' surface areas. Further studies on these tumors are therefore of special interest from ecological, pathogenic and cellular points of view.

Tumor formations in the massive coral species *Pavona clavus* (Jiménez 1998) at the northern Pacific coast of Costa Rica are strictly located to a single reef site (the Güiri-Güiri reef) in the Papagayo Gulf (Fig. 1); other reefs in the area do not show any indication of this phenomenon. The Güiri-Güiri reef is also the largest of the *P. clavus* reefs described so far from the eastern Pacific and has the highest "bioconstructional value" for the *Pavona* reefs of the Costa Rican region (Jiménez 1997). The reef is 220 m long surrounded by wide sandy areas (Fig. 1) and is almost exclusively composed by *Pavona clavus* (98% of the total coral cover; Jiménez 1997). Other rare coral species are *Pocillopora elegans* (1.6%), *Pavona gigantea* (0.2%), *Porites lobata* (0.2%) and *Pavona varians* (0.03%) (Jiménez 1997, 1998). Some *P. clavus* colonies have reached few meters in diameter and height, forming huge heads of several hundred years old. Although this is a common species in the region (Cortés 1997), we did not record a single recruitment in the three years of observation (Gateño, unpublished data). Here we followed *in situ* tumor growth and formation and studied tumorigenic tissues and skeletons for understanding this phenomenon. A major aim in this study is to classify the tumors developed on *P. clavus* and to identify biological characteristics of this phenomenon as revealed from the terms "tumor", "neoplasm" and "hyperplasia".

Materials and Methods

Study site. This study was carried out in the Güiri-Güiri reef between March 1998 and April 2001. The reef area is subjected to a yearly upwelling period (December – March) due to strong eastern winds in which a decrease of water temperature (28°C in June-July to 16°) is evident. Corals grow on sandy floors approximately 100 m off shore (Güiri-Güiri or Varador beach) and between 3 to 13 m depth (measured during low tide). The reef is about 220 m long by 50 m width in the SW-NE direction; comprising three distinctive parts that are separated from each other by sandy floors: the shallow reef (3~7 m depth), the intermediate reef (7~9 m

depth) and the deep reef (10~13 m depth). A small number of coral colonies that grow scattered on the sandy floor, are termed here as the sandy bottom reef (Fig. 1).

Morphology. Gross coral morphologies (at the colony and the polyp levels) were studied by several methodologies, including: direct field observations; *in situ* photography using a Nikonos V camera with a close-up extension tube; observations through dissecting microscopy and scanning electron microscopy. For scanning electron microscopy purposes, skeleton fragments were randomly sampled by chisel and hammer from 12 different colonies. We obtained, by the use of metal corers, 1 to 4 cores per fragment (total = 21), 10 mm in diameter each. Cores were cleaned with compressed air, glued to metal bases and photographed under the dissecting microscope. Then, they were covered with a 50 nm gold layer for observation under a scanning electron microscope (Hitachi S-2360).

Tumor frequency and distribution - field study. Observations were performed from November 1999 to October 2000. Line transects (n=18, six in each part of the reef) were run perpendicular to shore and all colonies underneath the line were included. For each colony, we documented: a) length, width and height of the colony; b) number of tumors per colony; c) length, width and height of each tumor; d) type of tumor and e) location of the tumor on the colony: top, directly exposed to the sunlight and possible fish predation; side, less exposed to light and predation; and bottom, at the most protected part of the colony.

Coral colonies and tumor structures were considered as half spheres and their surface areas (S) were calculated according to the formula $S = 2\pi r^2$, where r is the mean geometric radius of the height, width and length of the colony. Additionally, 14 tumors and 4 healthy skeleton fragments were collected randomly for studying skeleton density (D), where D was calculated by measuring fragment weight (w) and volume (v), using D=w/v.

Growth rates. Twenty two coral fragments (10 ~ 20 cm in diameter), each containing a healthy party and a tumor were randomly collected from large colonies, vitally stained overnight with Alizarin Red dye (10 mg l^{-1}), glued with epoxy to building blocks and left *in situ* for up to 2 years. Thereafter, the fragments were collected, the tissues were removed by a water jet and the skeletons were cut with an electric saw perpendicular to the tumors. Measurements (1 to 5 per sample according to the evenness of growth rates in the different parts of the colony) were taken from the alizarin line to the periphery of the coral. Mean growth rates were calculated for each healthy and tumor section.

Light histology and TEM. Small coral fragments were collected for light and transmission electron microscopy and brought alive to the laboratory. They were placed overnight in an aquarium and fixed for 24 hours in a modified Karnovsky solution (glutaraldehyde 2.5%, paraformaldehyde 2%, sucrose 5.0% in cacodilate 0.1M pH 7.4; Karnovsky 1965). Samples were then decalcified in HCl 10% and EDTA 0.7% solutions (Glynn et al. 1991). Staining was employed using Takahashi (1979) protocol. Polyps were embedded in 3% agarose (Bozzola & Russell 1992), dehydrated in increasing concentrations of ethanol, embedded in Spurr epoxic resin, sliced (85nm) and observed on the transmission electron microscope (Hitachi H-7100 at 100KV).

A total of 4 pairs of samples of *P. clavus* tumors and healthy parts were collected from the study site. Two additional samples of healthy tissue were sampled from a control reef, 5 km away, where *P. clavus* colonies did not express any sign of tumor formation.

Skeleton X-ray. Coral skeletons with tumors ($n = 20$) were sliced into thin plates (5mm width) and exposed to photographic plates in a Planmeca (Proline PM 2002) X-ray equipment at 60 KV, 12 mA for 0.3 seconds.

X-Ray element detection. Small pieces of coral skeleton were glued to metal bases and introduced to a Hitachi S-570 scan electron-microscope equipped with an X-ray detector

(Sirius 10/7.5, Res 133eV, Gresham Scientific Instruments, UK). The percentages of main elements presented in each sample were obtained. Coral samples were broken before mounting, so only the inner part of each sample was exposed and analyzed in order to avoid contamination. A total of 26 measurements were performed in two samples of healthy skeletons (n = 11) and four tumor samples (n = 15). The elements scanned were: C, O, Na, Mg, Sr, Si, P, S, Cl, Mn and Ca. The outcomes for Na and Cl were highly variable within and between samples, therefore they were not considered as part of the coral skeleton and were eliminated from the calculations.

In situ tissue contacts. Isogeneic (fragments from the same colony) and allogeneic (from different colonies) tissue-to-tissue contacts between healthy and tumor fragments were performed to investigate possible fusion-rejection reactions and transfer of infections to healthy fragments. We placed interactions from two coral genotypes in all 10 possible pairwise combinations: H1-H1; H1-T1; H1-H2; H1-T2; T1-T1; T1-H2; T1-T2; H2-H2; H2-T2; T2-T2 (where H = healthy tissue; T = tumor, and 1 and 2, are coral genotypes). The study was repeated 4 times with different pairs of colonies, making a total of 40 interactions. Fragments were glued *in situ* on building blocks with epoxy (Aquamend) and left there for two years.

Results

Corallite morphology. Regular *P. clavus* colonies have a characteristic uniform brown color morph, with the exception of regenerating areas damaged by fish predation. Corallites are well defined with an average surface area of 4.4 ± 0.8 mm^2 (n = 6) and total number of 10-22 thick septae (mean = 14.3 ± 3.2; n = 42). The primary septae reach the columella and are interleaved by thinner secondary septae that usually do not reach the polyp's center. Septa

carry granulations all over their surfaces, microstructurally consisting of ordered and relatively flat crystals (Figs. 2a, 3a-c).

Tumors in *P. clavus* are conspicuous formations, easily observed over the colony surface as presenting unusual phenotypic expression (Fig. 2). In many cases, tumor tissue has lighter coloration than the rest of the colony, similar to an earlier report (Cheney 1975). Tumors are subjected to increased predation by fish and drilling indices by boring organisms, probably due to their raised shapes and their porous skeleton (1.73 g/cm^3; SD=0.72) as compared to a healthy skeleton (2.13 g/cm^3; SD=0.25; single tailed t-test, $p<0.05$, d.f. =20). Cheney (1975) obtained similar results.

Three morphologically distinct types of tumors were identified from field observations, from observations under the dissecting microscope and the scanning electron microscope, as follows:

a) Type I tumor: This type presents a chaotic morphological state with no distinct structural organization of the skeletal corallite and a considerable diminution in the coloration, due to smaller numbers of zooxanthellae (Fig. 2b). The mean polyp area (3.3 ± 0.7 mm^2; n = 2), although smaller than the regular polyp, is not significantly different (Multiple comparison Bonferroni test; $p>0.05$). The septae are variable in number, shape and usually very thin (Figs. 2b, 3d-f). Columellae may be present or absent.

b) Type II tumor: This type of tumor reveals normal-like corallites of insignificant larger sizes (7.7 ± 2.7 mm^2; n = 8; Multiple comparison Bonferroni test; $p>0.05$), and presents the typical polyp coloration of this species. Columellae and septal arrangement are similar to healthy corallites (Figs. 2c, 3g-i).

c) Type III tumor: This type of tumor has a slightly lighter color morph than the healthy tissue. Corallites present very thick fenestrated primary septae with very thin secondary

septae. Some septae exhibit chaotic structures with porous skeletal formations. Columellae are present (Figs. 2d, 3j-l). The average corallite size (10.4 ± 4.4 mm^2; n = 7) is significantly larger than normal and tumor type I polyps (Multiple comparison Bonferroni test; $p<0.01$).

We counted septae numbers that were seen in dissecting microscopic photographs taken from 149 polyps (42, 13, 82 and 12 polyps of healthy, type I, II and III tumors, respectively). Means ± SD: 14.3 ± 3.2, 15.8 ± 3.3, 16.7 ± 3.0, 9.9 ± 1.3 for the healthy and tumor types I, II, III polyps, respectively. Type III tumors possess the least number of septae than the two other types ($P<0.05$) and significant differences were found between the healthy polyps and type II and III tumor polyps (ANOVA, $F = 20.777$, $df = 145$, $p<0.01$; Multiple comparison Bonferroni test; $p<0.01$). No significant differences were found between tumors types I and II.

Tumor frequencies and distribution. A total of 161 colonies (50 in the shallow reef; 52 in the intermediate reef; 50 in the deep reef; 9 on the sandy bottom; Table 1) were analyzed. Mean colony surface area in the Güiri-Güiri reef was 3.26 m^2. However, the average colony size in the intermediate zone population was significantly higher (7.7, 18.9 and 2.6 times) than the shallow, deep and sandy floor populations, respectively (Table 1).

More than half (54.7%; Table 1) of the *Pavona clavus* colonies in the Güiri-Güiri reef developed tumors. The percentage of colonies with tumors increased with depth, from 36% in the shallow reef, to 54% in the intermediate reef and to 74% in the deep reef. A similar gradient was found in the mean number of tumors per colony, 1.8, 3.0 and 6.4 in the shallow, intermediate and deep populations, respectively. When only diseased colonies were considered, the mean number of tumors per diseased colony increased to 5.0, 5.5 and 8.6, respectively. The maximum number of tumors found in a single colony was 57, in a colony from the deep reef population. The sandy bottom reef presented similar numbers of diseased

colonies as in the shallow reef, but revealed the lowest average tumor per diseased colony (2.6 ± 2.3; Table 2).

Tumors of the different types were not equally distributed. Type II tumors were the most common (59.7%), followed by type III (23.8%) and type I (16.5%). This pattern of tumor distribution was recorded in all reef areas, with the exception of the intermediate reef zone where more type I tumors than type III were found.

Colony size was not found to have a significant impact on the number of developed tumors (linear regression; df = 86; p>> 0.05; R^2= 0.01); however, colony size significantly affects the total accumulated tumor surface areas (linear regression; df = 86; p < 0.01; R^2 = 0.11). Tumors also varied in size, from an area of a few square millimeters and up to 37.5 m^2, covering the whole colony surface area (Table 3).

Out of 397 tumors studied, most (97%) were located in the top and the side parts of the colonies (Table 4). Type I tumors appeared almost exclusively at the top of the colonies (85%), while type II tumors were equally distributed between the colony's top and side. Type III tumors appeared mostly in the side parts of the colonies (79%). The bottom parts of the colonies were less infected by tumors revealing 6.2% type III tumors frequency, more than twice than the other tumor types.

Growth rates. Linear growth rates were calculated from tumors and healthy parts of 22 colonies. Following two years *in situ*, tumor linear growth extension was higher than regular parts, 9.9 ± 3.1 mm yr^{-1} as compared to 6.1 ± 2.9 mm yr^{-1}, respectively (ratio 1.86; paired t-test; p < 0.01). Tumor growth orientation was in three dimensions. As a result, the tumor mass gradually overgrew the adjacent healthy polyps (Fig. 4a, c, e).

By using the linear growth rates (LG) and the average density (D) of healthy and tumorigenic skeletons (D), the calcification rate (CR) was calculated in grams of deposition

per year per cm^{-2} surface area [CR=LG (cm y^{-1}) x D (g cm^{-3})]. Tumorigenic calcification rate (2.8 ± 1.4 g yr^{-1} cm^{-2}) was significantly higher (single tail t-test; d.f. = 42; $p < 0.05$) than normal calcification rate (2.0 ± 1.4 g yr^{-1} cm^{-2}).

Skeletal X-ray. A detailed analysis of the radiographs (Fig. 4b, d, f) reveals that tumors initiated from an area corresponding to a single polyp that has started to divide faster than neighboring polyps, producing a higher number of abnormal polyps. The tumorigenic skeletons represent less dense skeletal accretion than the normal skeleton. This outcome is exhibited even more in areas where the tumorigenic skeleton began overgrowing normal skeletons, indicating in the radiographs as white or pale areas as opposed to the darker fingerprint of normal skeletal X-rays. As in the normal corallite, annual dense growth bands are also seen in the tumorigenic parts, representing the growth during the winter, that is the dry and upwelling season in this area. The skeletal weight percentages of C, O and Mg were significantly higher in the tumorigenic samples than in the healthy ones, while the weight percentage of Ca was lower in normal samples as compared to tumors (Table 5). The other elements did not show any significant difference. Values for Ca/Mg were higher in normal skeletons (382.3) as compared with tumors (121.8) while Mg/Sr and Ca/Sr values were lower (0.5 vs. 1.0 and 76.2 vs. 81.0, respectively).

Light histology and TEM. Light and transmission electron microscopies did not reveal any cytological difference between the tumor and healthy tissues. Tissue organization, cell, nuclei and other organelle morphologies, were similar in both types of tissues without any sign of tissue atrophy and necrosis or high percentages of dividing cells. We could not find as well other diagnostic alternations to coral calicablastic epitheliomas (Peters et al. 1986) such as thinning of tissue in the tumor mass, loss of mucus, secretory cells or an increase in gastrovascular canals (Fig. 5). In both types of tissues, however, we found nuclei with

irregular structures (Fig. 5e, f). These types of irregularities were recorded not only in healthy and tumorigenic tissue sampled from colonies living in the Güiri-Güiri reef but also in tissues of control corals sampled from far away reefs where tumors are not found. The only difference observed was the appearance of large amounts of phagocyted and partially digested zooxanthellae in the tumor tissue (Fig. 5a, b). The endodermal layers in both types of tissues possessed large vacuoles, probably containing glycogen. Parasitic unicellular organisms, trematodes and infesting bacteria or algae were not observed. Cross sections (the soft tissue) of tumors always displayed complete polyps.

Interactions upon contact. The four sets of experiments revealed similar results. Fusions between fragments occurred only in isogeneic interactions, either in tumor vs. tumor, tumor vs. normal or normal vs. normal combinations. The contact zones between fragments in all allogeneic combinations were characterized by standoff positions. In one case, the tumor overgrew the normal tissue. Allogeneic interactions did not develop necroses and tumorigenic partners did not induce tumors in the healthy fragments (Table 6).

Discussion

Skeletal tumors in corals, termed as "calicoblastic epithelioma" (Peters et al. 1986), have been reported from coral reefs worldwide, including Okinawa, Japan (Yamashiro et al. 2000), the Hawaiian Islands (Squires 1965a, b, Hunter & Field 1997), The Netherlands Antilles (Bak 1983), Guam and Enewetak (Cheney 1975), Gulf of Oman (Coles & Seapy 1998), the Great Barrier Reef (Loya et al. 1984), French Polynesia (Le Campion-Alsumard et al. 1995), Jamaica (Wells 1973), Panama, Florida Keys, New Caledonia, El Ghardaqa (Red Sea), Truk Islands, Maldive Islands, British Virgin Islands, Belize (Peters 1986), Midway Islands (Cairns 1984) and more. The present study reveals the occurrence of skeletal tumors in the Pacific coast of Costa Rica, restricted to a single locality. At this locality (the Güiri-Güiri reef at

Bahia Culebra), the largest *Pavona clavus* reef described in the eastern Pacific (Jiménez 1997), more than half of the population carries calicoblastic tumors and many colonies express more than a single tumor, up to 57 tumors per colony. On the average, tumors have been found to cover 6% of the total surface areas of the diseased colonies.

This study further reveals that tumors in *P. clavus* are extravagant structures that start from a single polyp. Three morphologically distinctive types of tumors were found, characterized by different forms of abnormal polyp structures. The faster growth rate of the tumors, the lower skeletal density that they form, the light pigmentation of the tissue (algal lost) and the associated high infestation by boring organisms clearly distinguish morphologically tumor structures from the rest of the colony.

We found a gradient in tumor frequencies from the shallow to the deep reef but no apparent environmental factors were associated with this gradient. Water temperatures that were on the average only 0.3 °C warmer in shallow than in the deep reef or lower radiation in the deep reef could not be considered plausible causes for the high incidence of tumors in the deep population. Penetration of solar UV-B radiation in shallow tropical waters (Dunne & Brown 1996), therefore, may not induce tumors in *P. clavus*, as suggested for other coral species (Loya et al. 1984).

Tumors grew faster than benign skeletons. However, previous measurements in healthy *P. clavus* colonies (Jiménez 1998) revealed higher growth rate values. *P. clavus* in the Güiri-Güiri reef grew on the average 20.6 mm yr^{-1} during 1995-1996 (during 1998-2001, our results are 6.6 mm yr^{-1} and 9.9 mm yr^{-1} for normal and tumorigenic skeletons, respectively) and 13.2 mm yr^{-1} in Caño Island, in the southern Pacific coast of Costa Rica. Although these variations may reflect genuine differences, they may also reflect the different methodologies used. In our study, coral fragments were glued to building cement blocks that were placed on sandy

bottom. In the Jiménez (1998) study, corals were tied to metal bars 1 m above the seafloor. It is possible that the differences in growth rates resulted from reduced sedimentation impacts (Sebens 1991).

Tumors or neoplasms are usually defined by the gradual increase in the number of dividing cells that create a growing mass of tissue. As more and more of these dividing cells accumulate, the normal organization of the tissue gradually becomes disrupted, making the tumorigenic mass morphologically distinctive. Among the traits that distinguish tumorigenic tissues from normal developing tissues are the existence of a large number of dividing cells, variation in nuclear size and shape, variation in cell size and shape, loss of specialized cell features, loss of normal tissue organization, and a poorly defined tumor boundaries (Peters et al. 1986). None of these criteria was found here. We could not find, as well, any external agent (such as algae; Le Campion-Alsumard et al. 1995 and cited references) or intracellular causative agents (such as microbial infestation). Instead, we observed a more rapid coral skeletal growth, a faster polyp division, a variation of polyp size and shape and loss of the species typical polyp organization. We cannot speculate as well as to whether the tumors may metastasize since even iso-fusions between normal and tumor parts did not evoke, after two years, tumors in healthy parts. Tumors in *P. clavus* appear to be localized and non-epidemic, possessing all components of the Cnidarians two germ layers (with the exception of the reduced number of zooxanthellae). They, however, produce morphologically abnormal polyps, that importantly, are similar to each other. The largest tumor in Güiri-Güiri had $37.5m^2$ tissue area covering the entire colony surface area with tumorigenic polyps. This tumor has probably developed for many years without any sign of necroses. It is possible, therefore, that the terminology used as "hyperplasia", "tumor" and "neoplasm" (Squires 1965a, Loya et al. 1984, Peters et al. 1986, Hunter & Field 1997, Yamashiro et al. 2000; all related to the soft tissue) do not represent the pathogenesis and etiology of this phenomenon in

P. clavus. The terms "calicoblastic epithelioma" (Peters et al. 1986; although referring to pathogenesis in the calicoblastic epithelium, an argument that was not supported by any published material) or "hard tissue tumor" (Cheney 1975) better represent the nature of this phenomenon.

Furthermore, the tumorigenic phenomenon in *P. clavus*, is an event that programs new pattern formation of polyps and colonies. The tumor begins as a single polyp, is irreversible in its structure, even following years of development and does not represent any cytological change. It grows faster than a normal part of the colony, does not infect contacting healthy tissues and does not carry life-threatening fates. This type of coral tumorigenic appearance is therefore distinct from the etiology and prognosis associated with the term "disease". The tumorigenic mass may, however, reciprocally interact with normal tissue on the physiological level. A nutrient depletion the healthy tissue may be developed by energy translocation through the gastrovascular system that connects between polyps (Gladfelter 1983a, Gateño et al. 1998). In fact, such an indication was revealed by Cheney (1975) as reduced growth rates in areas next to tumors.

Coral tumors are associated with calcification disorders. While Bak (1983) did not find changes in mineral composition, *P. clavus* tumorigenic skeletons clearly show differences in Mg concentration as well as skeletal density. These outcomes resemble Gladfelter (1983b) results showing different Mg concentrations in young vs. old skeletal areas of *Acropora cervicornis* colonies. It is possible, therefore, that the tumorigenic skeleton in *P. clavus* represents a "rejuvenalization" process of calcification, production of crystals similar to primary polyps (Vandeermeulen & Watabe 1973). We may further speculate that changes in gene expressions in the calicoblastic layer, which are associated with calcium carbonate deposition pathways, are the direct cause for the skeletal tumors in reef corals. This argument

is further supported by the fact that tumorigenic polyps are similar in structure to each other and are clearly distinguished from the species-specific morphologies (Cheny 1975, Bak 1983, Loya et al. 1984, Peters et al. 1986, Coles & Seapy 1998, Yamashiro et al. 2000, this study). These gene expression changes may reflect epigenetic phenomena, *de novo* changes in the genome (mutational hot spots, etc.), or any combinational changes of both types. Further studies on the calicoblastic layers of tumorigenic vs. normal tissues would probably reveal the nature of skeletal tumors in hermatypic corals.

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Table 1. General characteristics for tumor formations in *Pavona clavus* in the Guiri-Guiri reef. "N", are the number of colonies observed, "SD" are standard deviations, "range" reveals the minimum and the maximum values and "max" depicts the maximum values.

		Colony Surface Area (cm^2)		Population Characteristics				Diseased Colony Characteristics	
					Tumor frequency		Tumor Cover (%)	Tumor frequency	Tumor Cover (%)
Location	N	Mean ± SD	Range	Diseased Colonies (%)	Mean ± SD	Max	Mean ± SD	Mean ± SD	Mean ± SD
Shallow reef	50	4,776 ± 4,631	555 - 30,299	36.0	1.80 ± 4.4	24	0.73 ± 1.93	5.00 ± 6.21	2.03 ± 2.8
Intermediate reef	52	89,678 ± 17,2507	447 - 567,684	53.9	2.96 ± 4.9	25	4.59 ± 18.3	5.50 ± 5.57	8.51 ± 24.5
Deep reef	50	2,920 ± 2,455	335 - 16,191	74.0	6.38 ± 11.1	57	5.28 ± 14.0	8.62 ± 12.12	7.14 ± 15.9
Sandy bottom	9	2,645 ± 2.7	1,528 - 86,033	55.6	1.44 ± 2.1	6	0.05 ± 0.1	2.60 ± 2.30	0.08 ± 0.1
Total	161	32,634 ± 29834	335 – 567,684	54.7	3.58 ± 7.43	57	3.35 ± 13.1	6.55 ± 9.04	6.03 ± 17.3

Table 2. Percentage of tumors by type in the four studied Guiri-Guiri zones.

Numbers in parenthesis are the absolute number of tumors observed.

Reef	Tumor type I	Tumor type II	Tumor type III	Total
Shallow	11.11 (10)	60.00 (54)	28.89 (26)	100 (90)
Intermediate	33.77 (52)	37.66 (58)	28.57 (44)	100 (154)
Deep	10.03 (32)	69.91 (223)	20.06 (64)	100 (319)
Sandy bottom	7.69 (1)	69.23 (9)	23.08 (3)	100 (13)
Total	16.49 (95)	59.72 (344)	23.79 (137)	100 (576)

Table 3. Mean tumor numbers and surface areas by location and type of tumor. N is the number of measured tumors, SD the standard deviation; CV the coefficient of variability and the range includes the minimum and maximum records for each row.

Reef Location	Tumor Type	N	Mean tumor size ± SD (cm^2)		CV (%)		Range (cm^2)
Shallow	I	10	26.55	± 42.60	160.5	0.21	141.83
	II	54	27.11	± 71.78	264.8	0.21	509.78
	III	26	12.23	± 29.47	241.0	0.06	108.54
	Total	90	22.75	± 59.07	259.6	0.06	509.78
Intermediate	I	55	7,180.92	± 52,080	725.3	0.10	375,759.84
	II	55	104.71	± 351.83	336.0	0.20	2,115.09
	III	44	37.61	± 124.45	330.9	0.02	739.37
	Total	154	2,612.76	± 30,280	1,158.9	0.02	375,759.84
Deep	I	32	34.73	± 89.99	259.1	0.13	422.19
	II	223	29.68	± 152.44	513.6	0.13	1,648.56
	III	64	14.03	± 67.00	477.5	0.16	537.34
	Total	319	27.04	± 133.97	495.5	0.13	1,648.56
Sandy bottom	I	1	20.05	± 0.00	0.0	20.05	20.05
	II	9	5.04	± 4.15	82.3	0.16	11.62
	III	3	1.18	± 0.83	70.3	0.33	1.99
	Total	13	5.30	± 5.83	110.0	0.16	20.05
Total		576	717.20	± 15,662	2,183.8	0.02	375,759.84

94C

Table 4. Location of tumors in the colony by the type of tumor.

Tumor type	Location in the colony	Frequency	Percent
I (n=40)	Top	34	85.0
	Side	5	12.5
	Bottom	1	2.5
II (n=276)	Top	129	46.7
	Side	141	51.1
	Bottom	6	2.2
III (n=81)	Top	12	14.8
	Side	64	79.0
	Bottom	5	6.2
Total (n=397)	Top	175	44.1
	Side	210	52.9
	Bottom	12	3.0

Table 5. Average percentage of major elements in normal and tumorigenic skeletons (± SD); n is the number of samples analyzed. Single tailed t-tests were performed for each element. ns - no significant differences were found ($p>0.05$), *$p<0.05$, ** $p<0.01$.

Element	Normal (n=20)	Tumors (n=24)	t-Test (d.f.=42)
O	50.44 ± 3.15	55.18 ± 4.54	**
Ca	36.98 ± 5.53	30.07 ± 6.86	**
Mg	0.25 ± 0.19	0.43 ± 0.31	**
C	11.80 ± 1.99	13.58 ± 2.82	*
Si	0.05 ± 0.05	0.08 ±0.07	ns
P	0.01 ± 0.01	0.03 ± 0.05	ns
S	0.27 ± 0.06	0.32 ± 0.20	ns
Mn	0.05 ± 0.07	0.08 ± 0.10	ns
Sr	0.50 ± 0.10	0.44 ± 0.18	ns

Table 6. Isogenic and allogeneic combinations for interactions. H = healthy, normal tissue; T = tumor, and 1 and 2, are coral genotypes; "+" indicates a positive outcome for at least a single case out of the four sets of interactions.

		Major results		
Interaction		**Fusion**	**Necrosis**	**Infection**
H1-H1	Isogeneic	+	-	-
H1-T1	Isogeneic	+	-	-
H1-H2	Allogeneic	-	-	-
H1-T2	Allogeneic	-	-	-
T1-T1	Isogeneic	+	-	-
T1-H2	Allogeneic	-	-	-
T1-T2	Allogeneic	-	-	-
H2-H2	Isogeneic	+	-	-
H2-T2	Isogeneic	+	-	-
T2-T2	Isogeneic	+	-	-

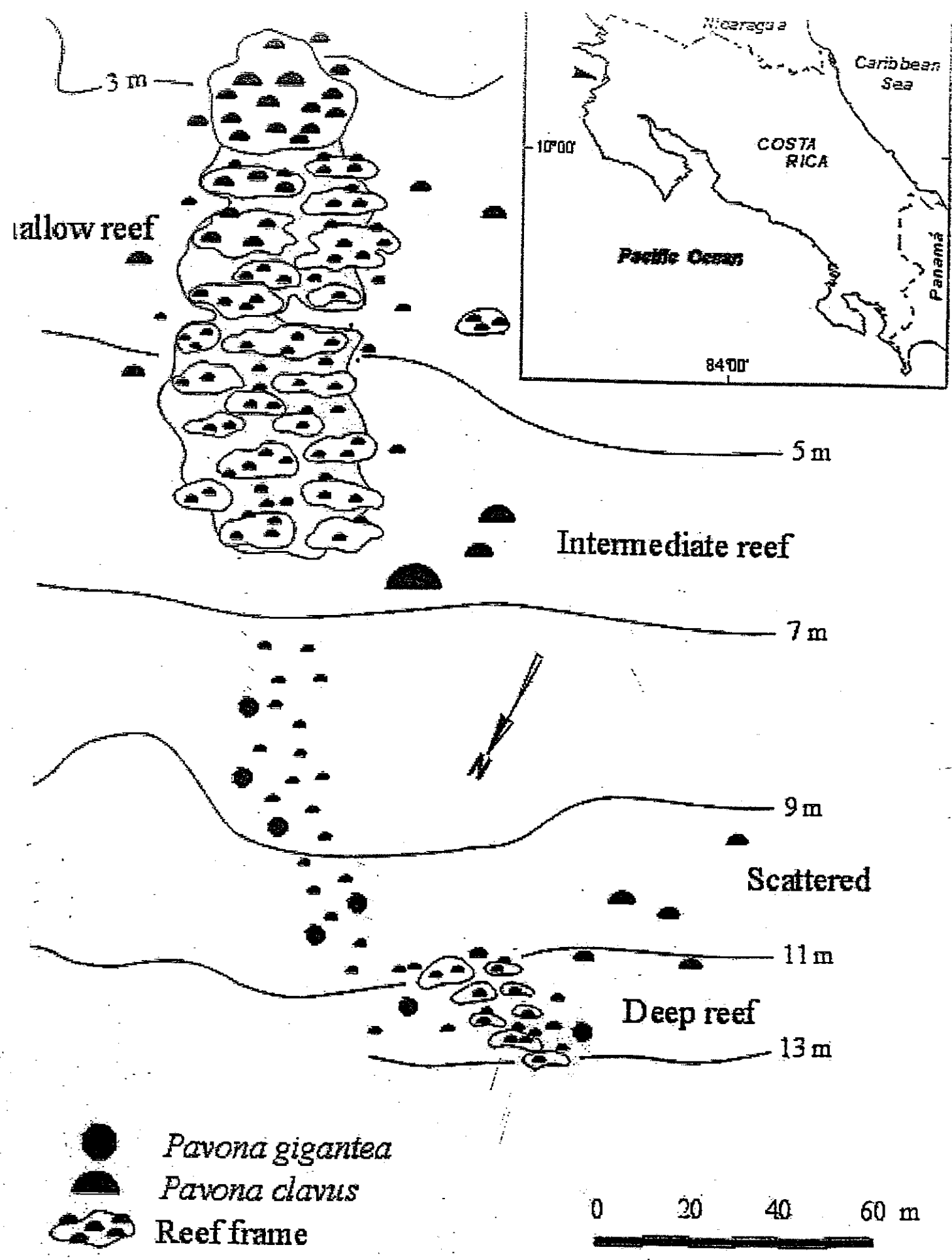
3 m
allow reef
Nicaragua
Caribbean Sea
10°00'
COSTA RICA
Pacific Ocean
Panamá
84°00'
5 m
Intermediate reef
7 m
9 m
Scattered
11 m
Deep reef
13 m
Pavona gigantea
Pavona clavus
Reef frame
0 20 40 60 m

Fig 1

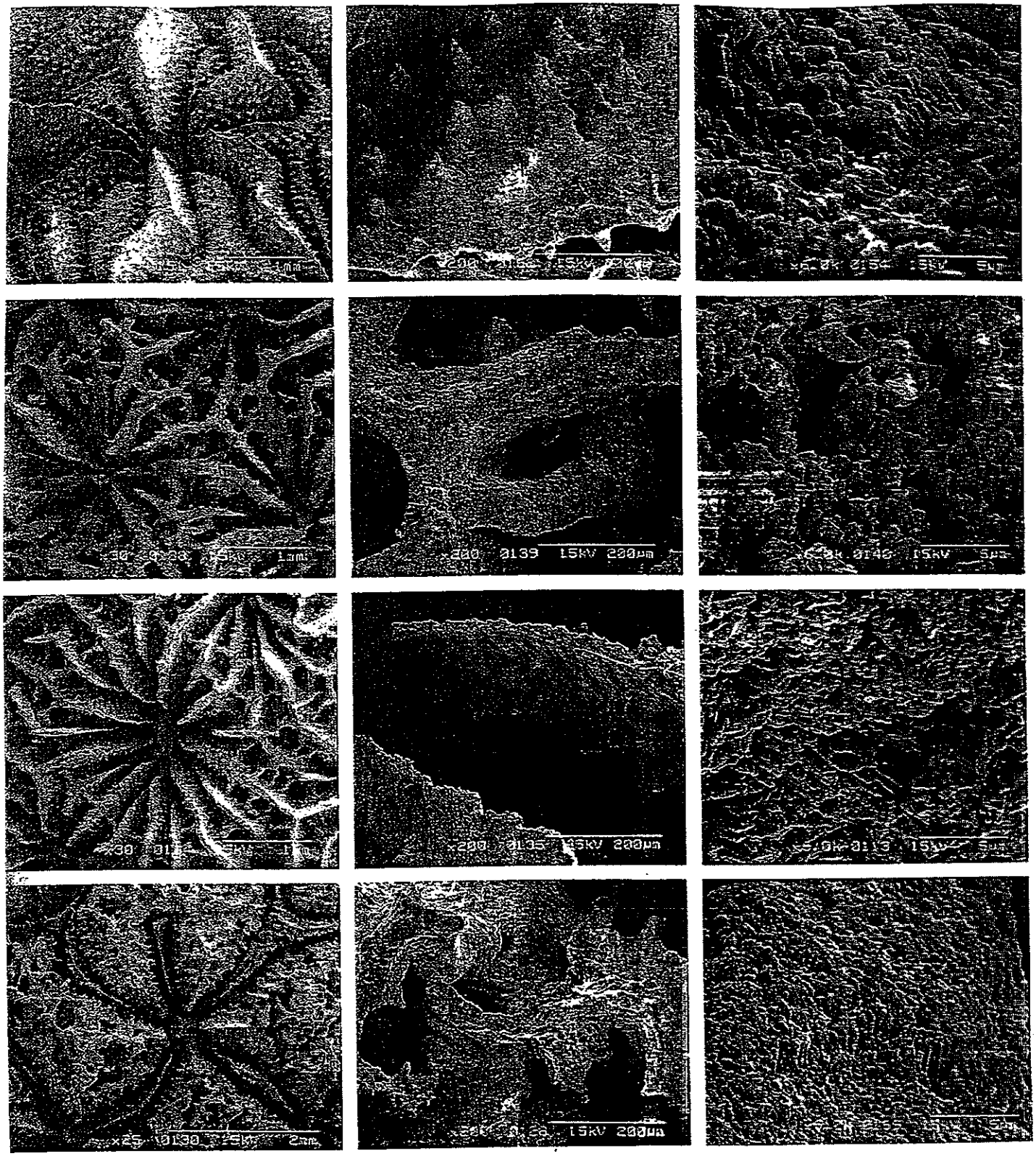

Fig 2

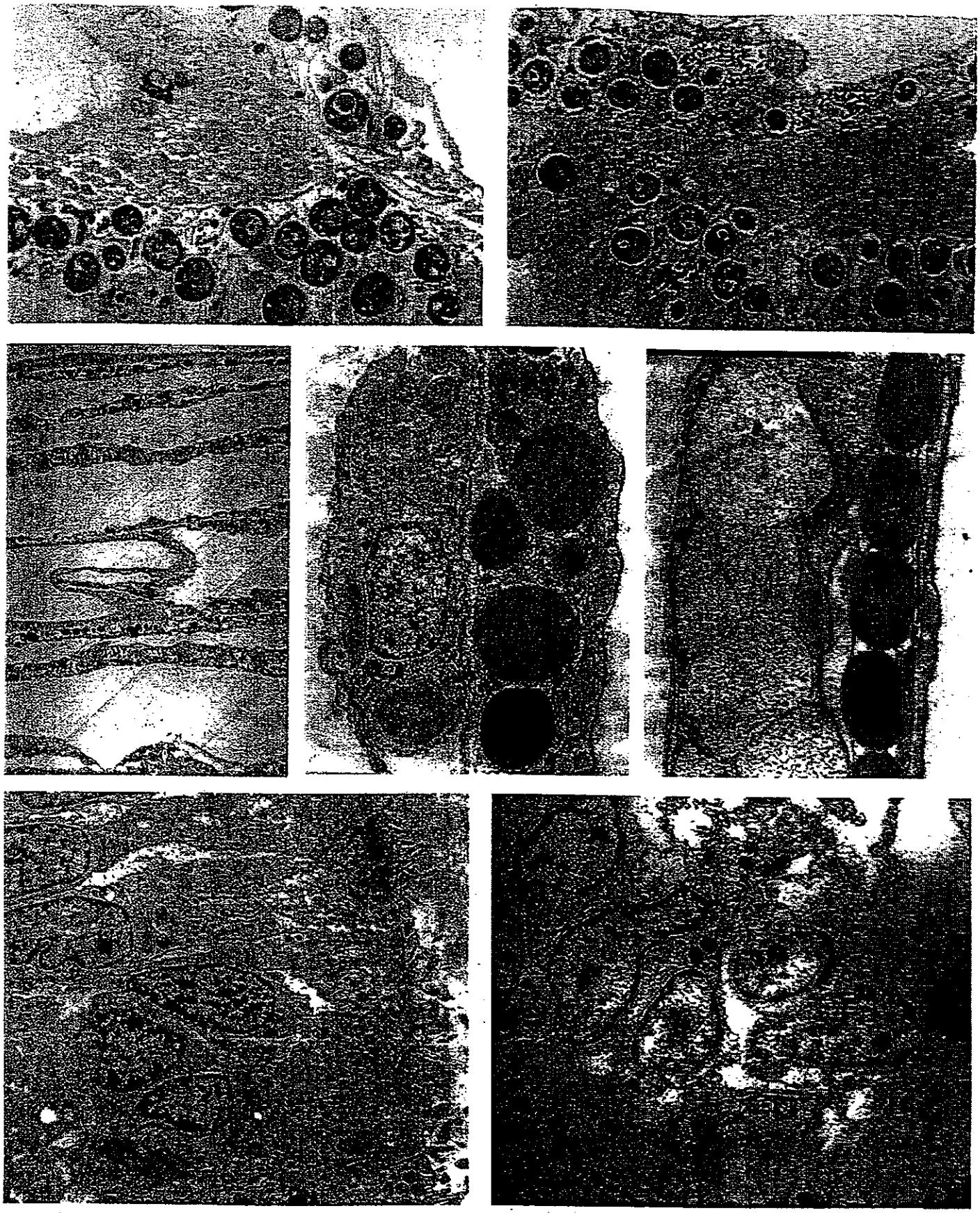

Fig 3

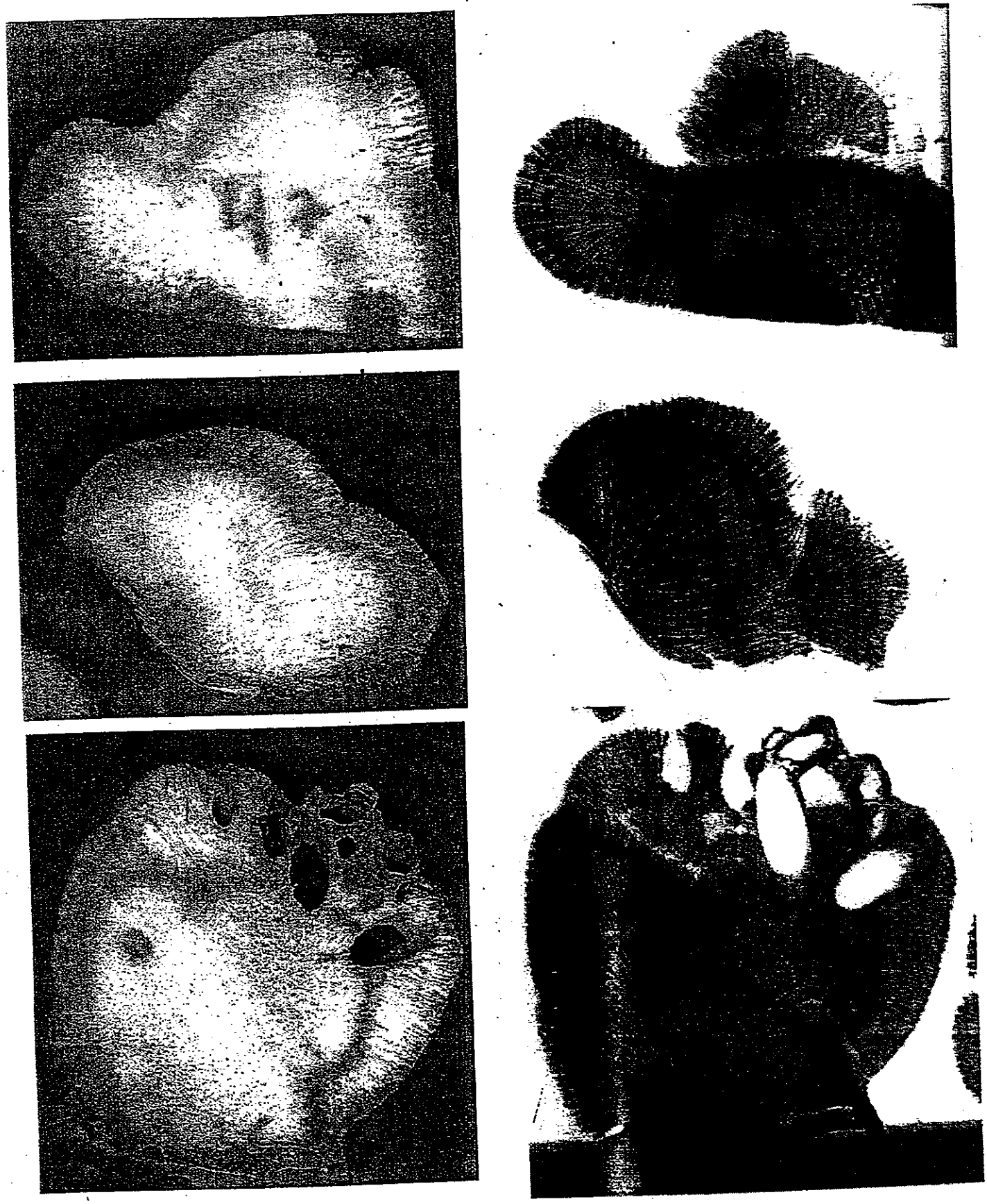

Fig 4

IMPACT RELEVANCE AND TECHNOLOGY TRANSFER

As mentioned above, the results of the projects have already been published in at least 14 manuscripts (2 during 1999, 4 during 2000, 4 during 2001, 2 during 2002 and at least 2 will be published in 2003). Detailed evaluation of "reef management policies", the concepts for reef domestication, reef restoration policies as developed from the studies and the other results obtained from the collaborative studies will help the decision making authorities for choosing better protocols in reef management. We have also started to evaluate genetic structures of coral populations and geometric characteristics of isolated branches and nubbins. This opens novel routes and opportunities for reef conservation. Detailed monitoring data received and the data anticipated in the future, will help us in understanding the year-to-year changes in Costa Rican coral reefs. This will establish a "data bank" of physical-chemical reef conditions. In addition, the data being generated has been used for several projects apart from this one: zooplankton distribution and seasonal changes in Bahía Culebra, the effects of El Niño in Bahía Culebra and paleoclimatic reconstructions. In addition, we anticipate that the identification of coral diseases (such as tumors) and the characterization of stress proteins from reef corals will enhance the understanding of the stressed reefs during their regular monitoring plans.

All technological achievements that were generated during this study were transferred to the Costa Rican partner. The mutual work of the two Israeli scientists residing in Costa Rica with the local scientific group and the recent visit of the Costa Rican team to Israel further strengthen the CIMAR scientific activities. The mutual studies that have been carried out provide the opportunity to transfer ideas, data, knowledge and specific scientific issues to local students. Several meetings with other scientists from CIMAR were already conducted, for the purpose of involving additional scientists in the projects and several close collaborative

study projects have been established between CIMAR and the University of Bremen, Germany.

PROJECT OUTPUTS

As specified, 14-peer reviewed manuscripts describing results of the work generated in this study, were already published or were accepted for publication, as follows:

1 Tom, M., Douek, J., Yankelevich, I., Bosch, T.C.G. and Rinkevich, B. Molecular characterization of the first heat shock protein 70 (HSP70) from a reef coral. Biochem. Biophys. Res. Commun. 262, 103-108, 1999.

2 Epstein, N., R.P.M. Bak and B. Rinkevich. Implementation of small scale "no-use zone" policy in a reef ecosystem: Eilat's reef-lagoon six years later. Coral Reefs 18, 327-332, 1999.

3 Barki, Y., J. Douek, D. Graur, D. Gateño and B. Rinkevich. Polymorphism in soft coral larvae revealed by amplified fragment-length polymorphism (AFLP) markers. Mar. Biol. 136, 37-41, 2000.

4 Gateño, D., Barki, Y. and B. Rinkevich. Aquarium maintenance of reef octocorals raised from field collected larvae. Aquarium Sci. Conservation 2, 227-236, 2000.

5 Rinkevich, B. and S. Shafir. *Ex situ* culture of colonial marine ornamental invertebrates: Concepts for domestication. Aquarium Sci. Conservation 2, 237-250, 2000.

6 Epstein, N., R.P.M. Bak and B. Rinkevich. Toxicity of 3rd generation dispersants and dispersed Egyptian crude oil on Red Sea coral larvae. Mar. Pollut. Bull. 40, 497-503, 2000.

7 Epstein, N. and B. Rinkevich. From isolated ramets to coral colonies: The significance of colony pattern formation in reef restoration practices. Basic Appl. Ecol. 2, 219-222, 2001.

8 Shafir, S., J. Van Rijn and B. Rinkevich. Nubbing of coral colonies: A novel approach for the development of island broodstocks. Aquarium Sci. Conserv. 3, 183-190, 2001.

9 Jiménez, C., J. Cortés, A. León and E. Ruiz. Coral bleaching and mortality associated with El Niño 1997/98 event in an upwelling environment in the eastern Pacific (Gulf of Papagayo, Costa Rica). Bulletin of Marine Science 69, 151-169, 2001.

10 Epstein, N., R.P.M. Bak and B. Rinkevich. Strategies for gardening denuded coral reef areas: The applicability of using different types of coral material for reef restoration. Restoration Ecol. 9, 432-442, 2001.

11 Rinkevich, B. The branching coral *Stylophora pistillata*: The contribution of genetics in shaping colony landscape. Israel J. Zool. 48, 71-82, 2002.

12 Paz, G., J. Douek, C. Mo, M. Goren and B. Rinkevich. Genetic structure of *Botryllus schlosseri* (Tunicata) populations from the Mediterranean coast of Israel. Mar. Ecol. Prog. Ser. (in press).

13 Gateño, D. and B. Rinkevich. Coral polyp budding is determined by a canalized ration of two morphometric fields. Mar. Biol. (in press).

14 Bongiorni, L., S. Shafir, D. Angel and B. Rinkevich. Survival, growth and gonadal development of two hermatypic corals subjected to *in situ* fish farm nutrient enrichment. Mar. Ecol. Prog. Ser. (in press).

Reprints of two papers are appended (nos. 10, 11). There are two more publications have already been submitted for publication and one manuscript that is now in preparation, bringing the total number of publications coming out from this AID-CDR project at least 17. The results of this project were also presented in scientific meetings. For example, funds were used to support Jorge Cortes at the IX Congreso Latinoamericano sobre Ciencias del Mar (COLACMAR), San Andres Isla, Columbia. Universidad Nacional de Colombia, Sede San Andres, 16-20 Septembe, 2001 (Latin American Congress on Marine Sciences). During this

meeting, two presentations as a result of the AID-CDR project were presented. In the same way, two oral presentations were given in the International Coral Reef Symposium held in Bali, 2000. Detailed presentations:

Environmental monitoring of a coral reef area, Bahia Culebra, Costa Rica. 9th International Coral Reef Symposium, Bali, October 23 – 27, 2000. Abstract page 343. (Poster with A. León.

Monitoring and status of coral reefs in the southern tropical America. 9th International Coral Reef Symposium, Bali, October 23 – 27, 2000. Abstract page 211. (Oral presentation: J. Garzón-Ferreira, J. Cortés, A. Croquer, H. Guzmán, Z. Leão and A. Rodríguez-Ramírez).

Los arrecifes coralinos de Costa Rica. IX Congreso Latinoamericano sobre Ciencias del Mar (COLACMAR), San Andrés Isla, Colombia. Universidad Nacional de Colombia, Sede San Andrés, 16 al 20 setiembre, 2001. Resumen página 72. (Oral presentation).

Monitoreo de arrecifes en el nodo América Tropical sur. IX Congreso Latinoamericano sobre Ciencias del Mar (COLACMAR), San Andrés Isla, Colombia. Universidad Nacional de Colombia, Sede San Andrés, 16 al 20 setiembre, 2001. Resumen página 73. (Oral presentation oral: J. Garzón-Ferreira, J. Cortés, A. Croquer, H. Guzmán, Z. Leão and A. Rodríguez-Ramírez).

The two publications from the 9th Coral Reef Symposium will be published as part of the Symposium publications. In addition to the above publication, a German student did his Master's thesis research at Bahía Culebra looking at the fisheries of ornamental fish. He found a decline in catch between 1994 and 2000. This decline may be due to a reduction in fishing effort because of the low profitableness of the activity. However, analysis of fish populations indicates that the fisheries can't expand any more because of possible over-fishing. His thesis title: Alperman, T.J. 2001. The fisheries of ornamental fishes in Guanacaste, Costa Rica, with

special emphasis on the population dynamics of the Cortez Rainbow wrasse, *Thalassoma lucasanum* (Gill 1863). M.Sc. Thesis, ISATEC, University of Bremen, Germany. 83 pages.

During the course of the project, we employed natural field studies, meetings, training and other activities, as follows:

Until December 1999 – : Two Israeli scientists (two postdoctoral fellows) have resided in Costa Rica, working together with the Costa Rican Team and were in direct collaboration contacts with the Costa Rican PI. An Israeli Ph.D. student is now working on the same questions in the Red Sea coral reefs and is analyzing some of the data collected from Costa Rican coral reefs.

During 1999, 2 field trips were conducted by the Israeli principal investigator to Costa Rica's reefs (on both sides, the Pacific and the Caribbean coasts). These trips which were conducted by both teams of scientists, continued the framework of mutual collaboration that was established in the specific areas studied. A third trip was performed by one of the Israeli postdoctoral fellows residing in Costa Rica (D. Gateño), traveling to the US for collaborative work and discussions with the Israeli PI on the research conducted.

We have selected areas in the Pacific (Bahia Culebra) and the Caribbean (Cahuita) coasts that were studied over the next years. From the Costa Rican side, two students have been selected to develop their thesis within the framework of the project. A Costa Rican technician has been collecting basic physical and biological data at the Pacific site. All studies/field trips were mutually inspected by both PIs.

Until December 2000 - : Dr. D. Gateño and Dr. Y. Barki, the two Israeli scientists (two postdoctoral fellows) continued to reside in Costa Rica working together with the Costa Rican team and were in direct collaboration contacts with the Costa Rican PI. The Israeli Ph.D. student continued working on the same questions in the Red Sea coral reefs and is analyzing some of the data collected from Costa Rican coral reefs (N. Epstein). During the fiscal year of

2000, 2 field trips were conducted by the Israeli principal investigator to Costa Rica's reefs (on both sides, the Pacific and the Caribbean coasts). These trips which were conducted collaboratively by both teams of scientists, continued the framework of mutual collaboration that was established in the specific areas studied. Two Costa Rican scientists (a Ph.D. student and a senior technician) visited the laboratory at Haifa this year, went for 2 weeks to the Red Sea for diving in the studied sites and were trained with techniques, the rationale and the aspects for reef restoration as developed in the laboratory at Haifa.

Until December 2001 – The Two Israeli postdoctoral fellows continued to reside in Costa Rica, working together with the Costa Rican team, as well as in direct collaboration contacts with the Costa Rican PI. A new Israeli Ph.D. student (S. Shafir) has stated to work on the same questions in the Red Sea coral reefs and is analyzing some of the data collected from Costa Rican coral reefs. During the fiscal year of 2001, one field trip was conducted by the Israeli principal investigator to Costa Rica's reefs (on both sides, the Pacific and the Caribbean coasts). This trip which was conducted collaboratively by both teams of scientists, continued the framework of mutual collaboration that was established in the specific areas studied. The two Costa Rican students finished their work and others were chosen to continue the work carried out on both sides (the Caribbean and the Pacific reefs. The Costa Rican technician who visited Israel last year is collecting basic physical and biological data at the Pacific site. All studies/field trips will be mutually inspected by both PIs. In addition, Carlos Jiménez, a Costa Rican Ph.D. student has been helped in his research on paleoclimate of the northern section of the Pacific coast of Costa Rica. This research will help decipher the intensity of passed upwellings, whose intensity is apparently is regulated by El Niño and maybe La Niña. A German student, Tilman Alperman, did his Master's thesis in the area and graduated this year. One trip to Costa Rica was activated, where the Israeli PI has visited the Costa Rican colleagues for one week, for discussions and trips to the field. As stated before, a tight collaboration has

been established on a day-to-day basis, as the result of the two Israeli postdoctoral fellows residing in Costa Rica. This is in addition to the direct contacts through e-mail, faxes and fast mails between the two laboratories.

Until December 2002 – The two Israeli postdoctoral fellows and the Israeli Ph.D. student continued their studies as before. One of the Israeli postdoctoral fellows (D. Gateño) gave a course at the University of Costa Rica on coral reefs and was involved in day-to-day discussions with the Costa Rican students and technicians. During the entire period of the mutual study (4 years), 8-10 field trips per year were conducted at which one of the Israeli postdoctoral fellows participated actively. One trip to Costa Rica was activated for one week for further discussions on the research outcomes and further plans. The Costa Rican Ph.D. student (C. Jiménez) will probably graduate during year 2003.

PROJECT PRODUCTIVITY/FUTURE WORK

The project scientifically accomplished a major part of the proposed goals. We now have on hand a long-term follow-up study on the environmental impacts (including sediment loads) on the corals along both coasts of Costa Rica. The detailed monitoring data received, will help us in understanding year-to-year changes in Costa Rican coral reefs. This will establish a "data bank" of physical-chemical reef conditions. The same applies for the restoration idea. While no formal plan has been formulated for the active restoration of the coral reefs in Costa Rica (due to the lack of funds at governmental levels), detailed evaluation of "reef management policies", the concepts for reef domestication, reef restoration policies as developed from the studies and the other results obtained from the collaborative studies will help the decision making authorities in Costa Rica for choosing better protocols in reef management. We have also started to evaluate genetic structures of coral populations and geometric characteristics of isolated branches. This opens novel routes and opportunities for reef conservation. Training,

another important key issue of the research, was successfully conducted during the entire course of the study. Technology transfer, direct day-to-day training, long-term side-by-side studies and collaborative work were enhanced by having two Israeli postdoctoral fellows residing in Costa Rica. These activities were based on matched funds which were added to the total $200,000 devoted for the purpose of the grant. These funds are limited. This is the reason why funds originally allotted for program expenditures until April 30, 2003 had already been depleted by the end of the fiscal year of 2002.

We are planning to continue this collaborative study in a new AID-CDR proposal which will focus on the results obtained from this study. Unfortunately, Costa Rica is no longer eligible as a primary target country. The study is highly relevant also to all other Central American countries (which are eligible countries for support). However, in neither one of the other Central American countries can such collaborative studies be developed due to the lack of facilities and trained scientists. Our request is to accept CIMAR (the cooperative Costa Rican marine institute) as a representative institute for a study which is relevant to the whole Central American region was not accepted. As such, a continuation of this activity by an AID-CDR project is no longer available. We are, however, planning to continue this type of collaboration through other avenues, such as the INCO Program (a European community collaborative program with developing countries). Project productivity will clearly be enhanced if the funds available for partners could be increased, especially for long-term studies (more than 3 years), as revealed in the present study.